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Search Results -

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LENTIVIRAL.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	249
LENTIVIRALLY.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	3
LENTIVIRAL-BASED.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	4
LENTIVIRAL-DERIVED.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	1
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LENTIVIRAL-PROTEIN.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	1
LENTIVIRAL-SPECIFIC.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	1
LENTIVIRAL-TRANSDUCED.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	1
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L4	L3 and HSC	0	L4
L3	L2 and lentivira$	2	L3
L2	l1 and fibronectin	2	L2
L1	RD114	29	L1

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=> s RD114
L1       244 RD114

=> s l1 and vector?
L2        61 L1 AND VECTOR?

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3        32 DUP REM L2 (29 DUPLICATES REMOVED)

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L3  ANSWER 1 OF 32  CAPLUS  COPYRIGHT 2002 ACS
AN  2001:676635  CAPLUS
DN  135:236393
TI  Highly efficient gene transfer into human repopulating stem cells by
      ***RD114***  envelope protein pseudotyped retroviral  ***vector***
    particles which pre-adsorb on retronectin-coated plates
IN  Kelly, Patrick F.; Vanin, Elio F.
PA  St. Jude Children's Research Hospital, USA
SO  PCT Int. Appl., 52 pp.
    CODEN: PIXXD2
DT  Patent
LA  English
FAN.CNT 1
    PATENT NO.      KIND  DATE          APPLICATION NO.  DATE
    --------------- ---- --------      ---------------- --------
PI  WO 2001066150   A2   20010913      WO 2001-US7212   20010307
      W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
         CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID,
         IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
         MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE,
         SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
         ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
      RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
         DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
         BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
    US 2001051375   A1   20011213      US 2001-801302   20010307
PRAI US 2000-187534   P   20000307
AB  The present invention relates to a method for efficiently introducing
    exogenous genes into stem cells, particularly human stem cells. The
    method optionally includes the steps of inducing the proliferation of
    target cells by pre-stimulation with cytokines and/or growth factors,
    followed by incubating these cells with  ***RD114*** -pseudotyped
     ***vector***  particles. In a specific embodiment, the  ***vector***
    particles are retronectin-immobilized or ultracentrifugation-concd.
    retroviral  ***vector***  particles pseudotyped with the feline
    endogenous retrovirus ( ***RD114*** ) envelope protein. The present
    invention further discloses a method for somatic gene therapy, which can
    be used for various therapeutic applications and involves introducing a
    gene of interest contained within the retroviral genome into human
    repopulating stem cells followed by introducing these cells into a human
    host. Finally, the present invention discloses a method for monitoring
    the efficiency of the stem cell-mediated gene transfer based on detecting
    the presence of the genes (or the expression products) of the retroviral
     ***vector***  in various stem cell-derived lineages of the host.

L3  ANSWER 2 OF 32  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN  2001:415218  BIOSIS
DN  PREV200100415218
TI  ***RD114*** -Pseudotyped oncoretroviral  ***vectors***  : Biological
    and physical properties.
AU  Kelly, Patrick F.; Carrington, Jody; Nathwani, Amit; Vanin, Elio F. (1)
CS  (1) Division of Experimental Hematology, Department of
    Hematology/Oncology, St. Jude Children's Research Hospital, 332 North
    Lauderdale, Memphis, TN, 38105: elio.vanin@stjude.org USA
SO  Orlic, Donald; Bruemmendorf, Tim H.; Sharkis, Saul J.; Kanz, Lothar.
    Annals of the New York Academy of Sciences, (June, 2001) Vol. 938, pp.
    262-277. Annals of the New York Academy of Sciences. Hematopoietic stem
    cells 2000: Basic and clinical sciences: Third International Conference.
    print.
    Publisher: New York Academy of Sciences 2 East 63rd Street, New York, NY,
    10021, USA.
    Meeting Info.: Conference on Hematopoietic Stem Cells: Genetics and
    Medicine Tubingen, Germany September 14-16, 2000
    ISSN: 0077-8923. ISBN: 1-57331-295-9 (cloth), 1-57331-296-7 (paper).
DT  Book; Conference
LA  English
SL  English

L3  ANSWER 3 OF 32  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
    1
AN  2001:526085  BIOSIS
DN  PREV200100526085
TI  Engraftment of NOD/SCID mice with human CD34+ cells transduced by
    concentrated oncoretroviral  ***vector***  particles pseudotyped with
    the feline endogenous retrovirus ( ***RD114*** ) envelope protein.
AU  Gatlin, Joel; Melkus, Michael W.; Padgett, Angela; Kelly, Patrick F.;
    Garcia, J. Victor (1)
CS  (1) Division of Infectious Diseases Department of Internal Medicine,
    University of Texas Southwestern Medical Center at Dallas, Y9.206, Dallas,
    TX, 75390-9113: victor.garcia@utsouthwestern.edu USA
SO  Journal of Virology, (October, 2001) Vol. 75, No. 20, pp. 9995-9999.
    print.
    ISSN: 0022-538X.
DT  Article
LA  English
SL  English
AB  Oncoretrovirus  ***vectors***  pseudotyped with the feline endogenous
    retrovirus ( ***RD114*** ) envelope protein produced by the FLYRD18
    packaging cell line have previously been shown to transduce human
    hematopoietic progenitor cells with a greater efficiency than similar
    amphotropic envelope-pseudotyped  ***vectors*** . In this report, we
    describe the production and efficient concentration of  ***RD114***
    -pseudotyped murine leukemia virus (MLV)-based  ***vectors*** .
    Following a single round of centrifugation,  ***vector***  supernatants
    were concentrated approximately 200-fold with a 50 to 70% yield.
    Concentrated  ***vector***  stocks transduced prestimulated human CD34+
    (hCD34+) cells with approximately 69% efficiency (n = 7, standard
    deviation = 4.4%) using a single addition of  ***vector***  at a low
    multiplicity of infection (MOI = 5). Introduction of transduced hCD34+
    cells into irradiated NOD/SCID recipients resulted in multilineage
    engraftment with long-term transgene expression. These data demonstrate
    that  ***RD114***  -pseudotyped MLV-based  ***vectors***  can be
    efficiently concentrated to high titers and that hCD34+ cells transduced
    with concentrated  ***vector***  stocks retain in vivo repopulating
    potential. These results highlight the potential of  ***RD114***
    -pseudotyped oncoretrovirus  ***vectors***  for future clinical
    implementation in hematopoietic stem cell gene transfer.

L3  ANSWER 4 OF 32  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
    2
AN  2001:512683  BIOSIS
DN  PREV200100512683
TI  Sustained multilineage gene persistence and expression in dogs
    transplanted with CD34+ marrow cells transduced by  ***RD114***
    -pseudotype oncoretrovirus  ***vectors*** .
AU  Goemer, Martin; Horn, Peter A.; Peterson, Laura; Kurre, Peter; Storb,
    Rainer; Rasko, John E. J.; Kiem, Hans-Peter (1)
CS  (1) Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D1-100,
    Seattle, WA, 98109-1024: hkiem@fhcrc.org USA
SO  Blood, (October 1, 2001) Vol. 98, No. 7, pp. 2065-2070. print.
    ISSN: 0006-4971.
DT  Article
LA  English
SL  English
AB  Previous studies have shown that the choice of envelope protein
    (pseudotype) can have a significant effect on the efficiency of retroviral
    gene transfer into hematopoietic stem cells. This study used a competitive
    repopulation assay in the dog model to evaluate oncoretroviral
     ***vectors***  carrying the envelope protein of the endogenous feline
    virus,  ***RD114*** . CD34-enriched marrow cells were divided into equal
    aliquots and transduced with  ***vectors***  produced by the
     ***RD114***  -pseudotype packaging cells FLYRD (LgGLSN and LNX) or by the
    gibbon ape leukemia virus (GALV)-pseudotype packaging cells PG13 (LNY). A
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total of 5 dogs were studied. One dog died because of infection before sustained engraftment could be achieved, and monitoring was discontinued after 9 months in another animal that had very low overall gene-marking levels. The 3 remaining animals are alive with follow-ups at 11, 22, and 23 months. Analyses of gene marking frequencies in peripheral blood and marrow by polymerase chain reaction revealed no significant differences between the ***RD114*** and GALV-pseudotype ***vectors*** . The LgGLSN ***vector*** also contained the enhanced green fluorescent protein (GFP), enabling us to monitor proviral expression by flow cytometry. Up to 10% of peripheral blood cells expressed GFP shortly after transplantation and approximately 6% after the longest follow-up of 23 months. Flow cytometric analysis of hematopoietic sub-populations showed that most of the GFP-expressing cells were granulocytes, although GFP-positive lymphocytes and monocytes were also detected. In summary, these results show that ***RD114*** -pseudotype oncoretroviral ***vectors*** are able to transduce hematopoietic long-term repopulating cells and, thus, may be useful for human stem cell gene therapy.

L3 ANSWER 5 OF 32 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 3
AN 2001240397 EMBASE
TI ***RD114*** -pseudotyped oncoretroviral ***vectors*** : Biological and physical properties.
AU Kelly P.F.; Carrington J.; Nathwani A.; Vanin E.F.; Stamatoyannopoulos G.; Dick J.E.; Eaves C.J.; Dunbar C.E.; Sharkis S.; Moore M.A.S.; Quesenberry P.J.
CS Dr. E.F. Vanin, Division of Experimental Hematology, Department of Hematology, St. Jude Children's Res. Hospital, 332 North Lauderdale, Memphis, TN 38105, United States. elio.vanin@stjude.org
SO Annals of the New York Academy of Sciences, (2001) 938/- (262-277). Refs: 49
ISSN: 0077-8923 CODEN: ANYAA
CY United States
DT Journal; Conference Article
FS 004 Microbiology
016 Cancer
022 Human Genetics
025 Hematology
029 Clinical Biochemistry
LA English
SL English
AB Limited functional expression of the viral envelope receptor is a recognized barrier to efficient oncoretroviral mediated gene transfer. To circumvent this barrier we evaluated a number of envelope proteins with respect to gene transfer efficiency into primitive human hematopoietic stem cell populations. We observed that oncoretroviral ***vectors*** pseudotyped with the envelope protein of feline endogenous virus (***RD114***) could efficiently transduce human repopulating cells capable of establishing multilineage hematopoiesis in immunodeficient mice after a single exposure to ***RD114*** -pseudotyped ***vector*** . Comparable rates of gene transfer with amphotropic and GALV-pseudotyped ***vectors*** have been reported, but only after multiple exposures to the viral supernatant. Oncoretroviral ***vectors*** pseudotyped with the RDI14 or the amphotropic envelopes had similar stability in vitro, indicating that the increased efficiency in gene transfer is at the receptor level likely due to increased receptor expression or an increased receptor affinity for the ***RD114*** envelope. We also found that ***RD114*** -pseudotype ***vectors*** can be efficiently concentrated, thereby removing any adverse effects of the conditioned media to the long-term repopulating potential of the target human hematopoietic stem cell. These studies demonstrate the potential of ***RD114*** -pseudotyped ***vectors*** for clinical use.

L3 ANSWER 6 OF 32 CAPLUS COPYRIGHT 2002 ACS
AN 2000:210402 CAPLUS
DN 132:247121
TI Pseudotyped retroviral ***vector*** gene transfer system for hemophilia in vivo gene therapy
IN Vandendriessche, Thierry; Chuah, Marinee K. L.
PA Vlaams Interuniversitair Instituut Voor Biotechnologie Vzw, Belg.
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000017375	A2	20000330	WO 1999-EP7384	19990921
WO 2000017375	A3	20000727		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9964681	A1	20000410	AU 1999-64681	19990921
PRAI EP 1998-203203	A	19980923		
WO 1999-EP7384	W	19990921		

AB The present invention relates to a gene transfer system, preferably pseudotyped retroviral ***vectors*** allowing stable expression of biol. active proteins at therapeutic, physiol. or supraphysiol. levels. The invention relates particularly to a method to treat hemophilia A or B using said ***vectors*** to express coagulation factors by in vivo gene therapy. Pseudotyping the retroviral ***vectors*** prevents induction of inhibitory or neutralizing antibody against the biol. active protein expressed in the animal model or the patient injected with the ***vector*** . VSV-G pseudotyped MFG-FVIIIDB retroviral ***vector*** was generated and injected i.v. into factor VIII (FVIII)-deficient mice. Long term, high level expression of human FVIII was detected in 6 of 13 mice, without the detection of human FVIII-specific inhibitory antibodies. These mice expressing a high level of human FVIII survived an otherwise lethal tail-clipping, demonstrating phenotypic correction of hemophilia A in FVIII-deficient mice.

L3 ANSWER 7 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2000:346887 BIOSIS
DN PREV200000346887
TI Precise gene localization by phenotypic assay of radiation hybrid cells.
AU Rasko, John E. J.; Battini, Jean-Luc; Kruglyak, Leonid; Cox, David R.; Miller, A. Dusty (1)
CS (1) Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Room C2-023, Seattle, WA, 98109-1024 USA
SO Proceedings of the National Academy of Sciences of the United States of America, (June 30, 2000) Vol. 97, No. 13, pp. 7388-7392. print.
ISSN: 0027-8424.
DT Article
LA English
SL English
AB A high resolution map of the human genome previously has been constructed by using the G3 panel of human/hamster radiation hybrid cell lines and >15,000 unique human genetic markers. By determining whether human DNA sequences are present or absent in each of the hybrids, localization of single genes may routinely be achieved at approxeq250-kb resolution. In this paper we have tested whether similarly precise localization might be achieved by phenotypic screening of the hybrids to facilitate positional cloning of unknown genes. We assayed the susceptibility of each of the hybrid cell lines to transduction by retroviral ***vectors*** bearing different retroviral envelope proteins that recognize receptors present on human but not on hamster cells. The results for each of the retroviral ***vectors*** were informative and allowed precise localization of the receptor genes for the ***RD114*** cat endogenous retrovirus, xenotropic murine leukemia virus, and type C feline leukemia virus. After cloning of the receptors for these retroviruses, we found that standard genotypic mapping by PCR gave results that were nearly identical to those from phenotypic mapping. These experiments show that precise gene localization by phenotypic assay of radiation hybrids is practical and was not appreciably impacted by the known instability of such hybrid cells. This technique should be applicable to many other human genes having discernible phenotypes in hamster cells and, with completion of the human genome project, will allow rapid identification of unknown genes on the basis of phenotype.

L3 ANSWER 8 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 2000:378692 BIOSIS
DN PREV200000378692
TI Analysis of 4070A envelope levels in retroviral preparations and effect on target cell transduction efficiency.
AU Slingsby, Jason H.; Baban, Dilair; Sutton, Julia; Esapa, Margaret; Price, Toby; Kingsman, Susan M.; Kingsman, Alan J.; Slade, Andrew (1)
CS (1) Oxford Biomedica (UK) Ltd., Medawar Centre, Robert Robinson Avenue, Oxford Science Park, Oxford, OX4 4GA UK
SO Human Gene Therapy, (July 1, 2000) Vol. 11, No. 10, pp. 1439-1451. print.
ISSN: 1043-0342.
DT Article
LA English
SL English
AB A number of stable producer cell lines for high-titer Mo-MuLV ***vectors*** have been constructed. Development has previously centered on increasing end-point titers by producing maximal levels of Mo-MuLV Gag/Pol, envelope glycoproteins, and retroviral RNA genomes. We describe the production yields and transduction efficiency characteristics of two Mo-MuLV packaging cell lines, FLYA13 and TEFLYA. Although they both produce 4070A-pseudotyped retroviral ***vectors*** reproducibly at >1 X 106 LFU ml-1, the transduction efficiency of unconcentrated and concentrated virus from FLYA13 lines is poor compared with ***vector*** preparations from TEFLYA lines. A powerful inhibitor of retroviral transduction is secreted by FLYA13 packaging cells. We show that the inhibitory factor does not affect transduction of target cells by ***RD114*** -pseudotyped ***vectors*** . This suggests that the inhibitory factor functions at the level of envelope-receptor interactions. Phosphate starvation of target cells shows a two-fold increase in Pit2 receptor mRNA and causes some improvement in FLYA13 virus transduction efficiency. Western blots show that FLYA13 viral samples contain an eight-fold higher ratio of 4070A envelope to p30gag than that of virus produced by TEFLYA producer cell lines. This study correlates overexpression of 4070A envelope glycoprotein in retroviral preparations with a reduction of transduction efficiency at high multiplicities of infection. We suggest that TEFLYA packaging cells express preferable levels of 4070A compared with FLYA13, which not only enables high-titer stocks to be generated, but also facilitates a high efficiency of transduction of target cells.

L3 ANSWER 9 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 2000:415630 BIOSIS
DN PREV200000415630
TI Highly efficient gene transfer into cord blood nonobese diabetic/severe combined immunodeficiency repopulating cells by oncoretroviral ***vector*** particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein.
AU Kelly, Patrick F. (1); Vandergriff, Jody; Nathwani, Amit; Nienhuis, Arthur W.; Vanin, Elio F.
CS (1) Division of Experimental Hematology, St Jude Children's Research Hospital, 332 N Lauderdale, Room D-4026, Memphis, TN, 38105 USA
SO Blood, (August 15, 2000) Vol. 96, No. 4, pp. 1208-1214. print.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB Limited expression of the amphotropic envelope receptor is a recognized barrier to efficient oncoretroviral ***vector*** -mediated gene transfer. Human hematopoietic cell lines and cord blood-derived CD34+ and CD34+, CD38- cell populations and the progenitors contained therein were transduced far more efficiently with oncoretroviral particles pseudotyped with the envelope protein of feline endogenous virus (***RD114***) than with conventional amphotropic ***vector*** particles. Similarly, human repopulating cells from umbilical cord blood capable of establishing hematopoiesis in immunodeficient mice were efficiently transduced with ***RD114*** -pseudotyped particles, whereas amphotropic particles were ineffective at introducing the proviral genome. After only a single exposure of CD34+ cord blood cells to ***RD114*** -pseudotyped particles, all engrafted nonobese diabetic/severe combined immunodeficiency mice (15 of 15) contained genetically modified human bone marrow cells. Human cells that were positive for enhanced green fluorescent protein represented as much as 90% of the graft. The use of ***RD114*** -pseudotyped ***vectors*** may be advantageous for therapeutic gene transfer into hematopoietic stem cells.

L3 ANSWER 10 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7

AN 2000:298131 BIOSIS
DN PREV200000298131
TI Efficient gene transfer into primary human CD8+ T lymphocytes by MuLV-10A1 retrovirus pseudotype.
AU Uckert, Wolfgang (1); Becker, Christian; Gladow, Monika; Klein, Dieter; Kammertoens, Thomas; Pedersen, Lene; Blankenstein, Thomas
CS (1) Max-Delbrueck-Center for Molecular Medicine, Robert-Roessle-Strasse 10, D-13092, Berlin Germany
SO Human Gene Therapy, (May, 2000) Vol. 11, No. 7, pp. 1005-1014. print. ISSN: 1043-0342.
DT Article
LA English
SL English
AB Efficient and stable gene transfer into primary human T lymphocytes would greatly improve their use for adoptive transfer to treat acquired disorders, viral diseases, and cancer. We have constructed retroviral ***vector*** pseudotypes of amphotropic murine leukemia viruses (A-MuLV, MuLV-10A1), gibbon ape leukemia virus (GaLV), and feline endogenous virus (***RD114***) containing the enhanced green fluorescent protein (GFP) as a marker gene. Transduction of primary human CD8+ T lymphocytes by the different GFP-retrovirus pseudotypes revealed the superiority of MuLV-10A1 in comparison with A-MuLV, GaLV, and ***RD114*** , respectively. The superior transduction efficacy of CD8+ T cells by MuLV-10A1 correlates with a longer half-life of this pseudotype in comparison with A-MuLV and, as shown by interference analysis with the human T cell line HUT78, by the utilization of both the A-MuLV receptor (Pit2) and the GaLV receptor (Pit1) for cell entry.

L3 ANSWER 11 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8
AN 2000:333399 BIOSIS
DN PREV200000333399
TI Transduction of human pancreatic tumor cells with vesicular stomatitis virus G-pseudotyped retroviral ***vectors*** containing a herpes simplex virus thymidine kinase mutant gene enhances bystander effects and sensitivity to ganciclovir.
AU Howard, Bradley D.; Boenicke, Lars; Schniewind, Bodo; Henne-Bruns, Doris; Kalthoff, Holger (1)
CS (1) Molecular Oncology Research Laboratory, Clinic for General and Thoracic Surgery, Christian Albrechts University, Arnold-Heller Str. 7, D-24105, Kiel Germany
SO Cancer Gene Therapy, (June, 2000) Vol. 7, No. 6, pp. 927-938. print. ISSN: 0929-1903.
DT Article
LA English
SL English
AB We examined the suitability of Moloney murine leukemia virus (MLV) 4070A-, cat endogenous virus (CEV) ***RD114*** -, or vesicular stomatitis virus G (VSV-G)-pseudotyped retroviruses containing the humanized enhanced green fluorescent protein (hEGFP) or one of two herpes simplex virus thymidine kinase (HSV-TK) genes to transduce and provide gene expression in human pancreatic tumor cells. Fluorescence-activated cell sorter analysis demonstrated that VSV-G-pseudotyped hEGFP ***vector*** infected a greater percentage of cells and generated more robust gene expression than MLV 4070A- or CEV ***RD114*** -pseodotyped ***vectors*** . Dot blot and Southern blot analysis of genomic DNA revealed up to 10-fold more gene copies in G418-selected VSV-G hEGFP ***vector*** -transduced cells compared with genomic DNA from cells transduced with MLV 4070A or CEV ***RD114*** pseudotypes. Cells transduced with VSV-G pseudotypes of HSV-TKWT or the HSV-TK30 ***vectors*** were 5- to 10-fold more sensitive to ganciclovir (GCV) than other pseudotype-transduced cells. A 40- to 61-fold difference in sensitivity to GCV was observed between cells transduced with VSV-G HSV-TK30 ***vector*** and cells transduced with MLV 4070A HSV-TKWT ***vector*** in vitro. A 13-fold reduction in tumor volume was observed in severe combined immunodeficient mice inoculated with PancTuITK30 cells compared with mice inoculated with PancTuITKWT cells during GCV treatment. We conclude that the choice of glycoprotein envelope and the potency of a particular suicide gene were therapeutically additive and increased the number of HSV-TK-positive cells and sensitivity toward GCV in human pancreatic tumors cells for prodrug gene therapy.

L3 ANSWER 12 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302193 BIOSIS
DN PREV200100302193
TI Multilineage transduction of non-human primate CD34+ hematopoietic cells using RD-114 pseudotyped oncoretroviruses.
AU Kelly, Patrick F. (1); Bonifacino, Aylin C.; Carrington, Jody A. (1); Agricola, Brian A.; Metzger, Mark E.; Kluge, Kim A.; Nienhuis, Arthur W. (1); Donahue, Robert E.; Vanin, Elio F. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 525a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The relative quiescence of the hematopoietic stem cell (HSC) and the low level of viral receptor expression are known to contribute to the low efficiency of retroviral gene transfer into HSCs of large animals and humans. We have previously reported that ***RD114*** -pseudotyped retroviruses could efficiently transduce cord blood CD34+ cells after 24-48 hours pre-stimulation and a single exposure to the viral particles preloaded onto RetroNectin-coated plates. Based on these results we evaluated gene transfer of ***RD114*** -pseudotyped murine retroviruses using non-human primate CD34+ peripheral blood (PB) cells in the rhesus autologous transplant model. SCF/G-CSF-mobilized rhesus monkey PB were collected and enriched for CD34+ cells. These cells were cultured in serum-containing medium with high concentrations of SCF, FLT-3 and IL-6 and exposed to ***RD114*** -pseudotyped particles preloaded onto RetroNectin-coated plates at 48 hours and 72 hours. After 96 hours in culture, cells were harvested and infused into irradiated recipients (2 X 500 cGy, n=5). The transduction efficiency of the infused cells was 35-55% based on EGFP expression. In all animals we have observed multilineage engraftment with persistence of EGFP expression after 6-8 weeks post-transplantation, a result that was not achieved with a similar construct pseudotyped with the amphotropic envelope protein. In the first animal transplanted, we observed high levels of multilineage engraftment of EGFP+ cells (as high as 98% in granulocytes) over the first 20 weeks post transplantation. After 26 weeks multilineage expression has stabilized at 8-10%. Serial genomic southern analysis for both proviral integrity and integration site indicated that ***vector*** silencing was not occurring and that the engraftment of gene modified cells was oligoclonal. The second recipient displayed similar kinetics but died from transplant related complications 8 weeks post-transplantation. Subsequent animals have achieved lower levels of EGFP expression (1-3%) suggesting that transduction conditions using this pseudotype remains to be optimized. These results suggest oncoretroviral ***vectors*** pseudotyped with the ***RD114*** envelope protein could be useful for achieving clinically relevant levels of gene transfer into human pluripotent hematopoietic cells.

L3 ANSWER 13 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:311867 BIOSIS
DN PREV200100311867
TI Improved transduction of human primitive hematopoietic cells with a lentiviral ***vector*** pseudotyped with the envelope protein of endogenous feline leukemia virus (***RD114*** .
AU Hanawa, Hideki (1); Kelly, Patrick F. (1); Nathwani, Amit C. (1); Nienhuis, Arthur W. (1); Vanin, Elio F. (1)
CS (1) Division of Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 524a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Conference
LA English
SL English
AB Lentiviral ***vectors*** based on HIV have inherent advantages in transducing non-dividing cells in that their pre-integration nucleoprotein complex is relatively stable and able to transverse the nuclear membrane without mitosis. Most HIV based ***vector*** systems studied to date have utilized the envelope protein of the vesicular stomatitis virus (VSV-G). We have found that the envelope protein of endogenous feline leukemia virus (***RD114***), when used to pseudotype murine oncoretroviral ***vectors*** , yields particles that very efficiently transduce primitive hematopoietic cells from cord blood, including those which establish human hematopoiesis in immunodeficient mice (Kelly et al, Blood 96:1206, 2000). Lentiviral ***vector*** particles pseudotyped with ***RD114*** envelope were produced by co-transfecting 293T cells with a ***vector*** plasmid which encodes the green fluorescent protein (GFP), a plasmid encoding the HIV matrix and enzyme proteins, a plasmid encoding the HIV tat and rev proteins, and either a plasmid encoding the VSV-G or ***RD114*** envelope protein. ***Vector*** production as assessed by p24 measurement in conditioned medium was essentially equivalent (VSV-G = 930ng/ml and ***RD114*** = 1240ng/ml). The titer of VSV-G particles was 30-fold higher on HeLa cells. At a multiplicity of infection (MOI) of 15 (HeLa titers) without prestimulation, transduction of cord blood CD34+ cells averaged 51.5% (range 15-76%) with ***RD114*** pseudotyped HIV ***vector*** particles whereas the corresponding values were 5.8% (range 2-9%) with the HIV ***vector*** pseudotyped with VSV-G o r less than 1% with murine oncoretroviral ***vector*** particles pseudotyped with ***RD114*** . With 48 hours of prestimulation, ***RD114*** pseudotyped lentiviral particles were more efficient than VSV-G pseudotyped particles at transducing cord blood (87% vs. 38%) or peripheral blood (51% vs. 21%) CD34+ cells. Using a second design, cells were exposed to equivalent numbers of ***vector*** particles based on p24 measurement. With this design, 72% of cord blood, CD34+ cells and 34% of CD34+, CD38- cells were transduced with ***RD114*** pseudotyped ***vector*** particles compared to 19% and 8%, respectively, with VSV-G pseudotyped lentiviral ***vector*** particles. Our results indicate that the ***RD114*** envelope will effectively pseudotype HIV based lentiviral ***vectors*** and suggest that ***RD114*** pseudotyped lentiviral ***vector*** particles transduce primitive human hematopoietic cells at greater efficiency than do VSV-G pseudotyped lentiviral ***vector*** particles.

L3 ANSWER 14 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:312397 BIOSIS
DN PREV200100312397
TI Retroviral mediated transfer of CD80 and CD86 into leukaemia cells: Investigating conditions for the optimum production of virus in a clinically relevant setting.
AU Browne, Sara J. (1); Blair, Allison (1); Rowbottom, Anthony; Pamphilon, Derwood H. (1)
CS (1) Bristol Institute for Transfusion Sciences, Bristol UK
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 2, pp. 376b-377b. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Conference
LA English
SL English
AB Acute lymphoblastic leukaemia (ALL), refractory to conventional therapy has been demonstrated to elicit a poor immune response in vivo. ALL cells have low expression of CD80 and CD86 costimulatory molecules and this may be partially responsible for the lack of an immune response to ALL cells in vivo. We aim to transfect ALL cells with CD80 and/or CD86 to produce anti-leukaemic T cells for use as a potential therapy for patients with disease refractory to conventional therapies. We wanted to develop a system of retroviral transfection in serum free medium (SFM) that could be adapted for clinical use. Constructs of CD80 and CD86 were made in the pBABEpuro and pBABEneo plasmids, respectively. The constructs were transfected into the K562 cell line by electroporation to ensure the genes could be expressed in human cells and detected. Both CD80 and CD86 were detectable by FACS analysis and shown to be highly expressed in clones selected in puromycin or neomycin (G418) containing medium (range of 50.73-99.89% cells from each clone expressed the transgene). CD80pBABEpuro and CD86pBABEneo were then transfected into the FlyRD18 feline retrovirus producing cell line, chosen because it has been shown to produce high viral titres in SFM and the ***RD114*** retroviral receptor is expressed at high levels in bone marrow. Transfection of K562 with these constructs demonstrated that both CD80 and CD86 could be expressed and detected by FACS analysis (range 63.66-99.16% cells from each clone expressed the transgene). CD80 and CD86 were expressed at significantly lower levels in the ***vector*** plasmid controls (puro and neo; $p<0.001$). In addition, we have tried to optimize viral titre by altering the conditions of viral production. We therefore investigated whether

removal of FBS increased viral titre in our culture system. In contrast to previous results, we found that removing FBS produced a minimum of 50% reduction in viral titre. Thus the production of virus may be dependent on the type of medium as well as the supplements added. We are now examining the use of human albumin solution (HAS) instead of FBS in virus production. Optimizing conditions for transfecting tumour cells is critical in generating transfectants with sufficient costimulatory activity to generate cytotoxic antitumour responses.

L3 ANSWER 15 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322016 BIOSIS
DN PREV200100322016
TI Comparison of three retroviral envelopes for high efficiency gene transfer into human marrow mesenchymal cells.
AU Hofmann, Ted J. (1); Capizzani, Tony R. (1); Kelly, Patrick F. (1); Vanin, Elio F. (1); Horwitz, Edwin M. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 220a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB Bone marrow stromal cell (MSCs) are marrow mesenchymal cells that are ideal vehicles for delivery of therapeutic proteins in gene therapy protocols. A major obstacle to any successful gene therapy strategy is obtaining high efficiency transduction of the target cells. To optimize transduction of MSCs for clinical trials, we compared the effect of the retroviral envelope on gene transfer efficiency. Three different pseudotypes of a murine stem cell viral ***vector*** , encoding the green fluorescent protein (GFP) as a marker, were produced: amphotropic (Ampho) in PA317 cells, GALV in PG13 cells, and ***RD114*** (RD) in FLYRD18 cells. The titer of each supernatant was determined using HeLa cells: Ampho = 4.1 X 104, GALV1 = 3.4 X 103, GALV2 = 1.2 X 105, and RD = 5.0 X 105 tu/ml. Following a standard 3-day transduction protocol, the human MSCs were analyzed by flow cytometry to determine the percentage of GFP positive cells. First, MSCs were transduced with Ampho (MOI = 0.2) yielding 92%; GALV1 (MOI = 0.02), 46%; GALV2 (MOI = 0.6), 68%; and RD (MOI = 2.5), 86% gene transfer. Next, MSCs were transduced with RD at an MOI of 0.2 (equivalent to Ampho) and 83% gene transfer was observed, not significantly different from the 86% transduction obtained using undiluted RD or the 92% obtained with Ampho. Finally, MSCs were transduced with either Ampho or RD at an MOI of 0.02 (equivalent to GALV1). Ampho transduced 77% and RD 61% of the MSCs, compared to 46% for GALV1. Notably, dilute RD (61%) and dilute Ampho (77%) transduced MSCs as well as the higher titer GALV2 (68%). Northern blot analysis showed an unexpected ratio (8:4:1) for the mRNAs of RDR (***RD114*** receptor), Pit-1 (GALV receptor), and Pit-2 (amphotropic receptor). Although RD and Ampho have similar potential to mediate gene transfer into MSCs, the mRNA for RDR is 8-fold more abundant than Pit-2 mRNA. Further, Pit-1 is 4-fold more abundant than Pit-2 despite the apparent lower gene transfer efficiency. We then compared the standard transduction of MSCs to transduction using RetroNectin coated dishes and found no difference in gene transfer efficiency. We conclude that amphotropic and ***RD114*** pseudotyped ***vectors*** are more effective for mediating gene transfer into MSCs. Further, more abundant receptor mRNA does not necessarily indicate a greater potential for transduction by the respective viral pseudotype. A higher titer GALV pseudotyped ***vector*** may be adequate for efficient transduction but sufficiently high titer PG13 supernatant has been difficult to generate. Additionally, RetroNectin does not enhance gene transfer in our system. Thus, ***RD114*** or amphotropic envelopes are preferred for clinical trials of MSC gene therapy.

L3 ANSWER 16 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322005 BIOSIS
DN PREV200100322005
TI Sustained multilineage gene persistence and expression in dogs transplanted with CD34+ marrow cells transduced by ***RD114*** pseudotyped oncoretroviral ***vectors*** .
AU Horn, Peter A. (1); Goerner, Martin (1); Peterson, Laura (1); Storb, Rainer (1); Kiem, Hans-Peter (1)
CS (1) Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 218a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB We have recently reported efficient gene transfer into canine hematopoietic repopulating cells using oncoretroviral ***vectors*** pseudotyped by the feline endogenous retrovirus envelope protein (***RD114***). Using a competitive repopulating assay in the dog model we compared gene transfer into hematopoietic stem cells between ***vectors*** produced by PG13 (GALV pseudotype) and FLYRD (***RD114*** pseudotype). CD34-enriched marrow cells from five dogs were divided into equal aliquots and transduced with LgGLSN (FLYRD), LNX (FLYRD) and LNY (PG13). All three ***vectors*** carried the neo gene and short sequence differences that allowed them to be distinguished in a single polymerase chain reaction. The ***RD114*** pseudotyped LgGLSN ***vector*** also contained the green fluorescent protein (GFP), enabling us to follow gene expression in transduced cells by flow cytometry. One animal died due to infection before sustained engraftment could be achieved and in the animal with lowest overall transduction rate follow-up was discontinued. We now present follow-up data of three dogs at 9, 18 and 21 months. Up to 10% of peripheral blood cells expressed GFP shortly after transplantation and up to 8% GFP-expressing cells were detected after 21 months. Flow cytometric analysis of hematopoietic subpopulations showed sustained GFP expression in all three dogs in DM5+ granulocytes, CD3+ lymphocytes and CD14+ monocytes. The percentage of GFP expressing cells was higher in granulocytes (up to 8.1%) than in lymphocytes (up to 3.5%) or monocytes (up to 5.7%). Two animals were examined for GFP expression in platelets and were found to have between 1.2-1.3% GFP+ platelets at 9 and at 21 months posttransplant. Since transduction efficiency has been shown to correlate with the level of retroviral receptor expression on target cells, we analyzed expression levels of the ***RD114*** receptor (RDR) on human and dog cells. Northern blot analysis revealed an almost 2-fold higher expression of RDR on human cells suggesting that human cells might be even more susceptible to transduction by ***RD114*** pseudotyped ***vectors*** than dog cells. In summary, our data show efficient transduction of canine hematopoietic repopulating cells using ***RD114*** pseudotyped retroviral ***vectors*** . The level of gene transfer and the sustained multilineage gene persistence and expression obtained in these experiments suggests that the ***RD114*** pseudotype is a promising alternative pseudotype for human stem cell gene therapy.

L3 ANSWER 17 OF 32 CAPLUS COPYRIGHT 2002 ACS
AN 1999:582627 CAPLUS
DN 131:195455
TI Retroviral ***vectors*** which are resistant to human complement inactivation and uses thereof in gene therapy
IN Pensiero, Michael; Collins, Mary K. L.; Cosset, Francois-Loic; Takeuchi, Yasuhiro; Weiss, Robin A.
PA Genetic Therapy, Inc., USA; Institute of Cancer Research Royal Cancer Hospital
SO U.S., 29 pp., Cont.-in-part of U.S. Ser. No. 291,765, abandoned. CODEN: USXXAM
DT Patent
LA English
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5952225	A	19990914	US 1995-516163	19950817
CA 2196208	AA	19960222	CA 1995-2196208	19950817
US 6329199	B1	20011211	US 1999-374746	19990813
PRAI US 1994-291765	B2	19940817		
US 1995-451215	B2	19950526		
US 1995-516163	A1	19950817		

AB The invention provides retroviral ***vectors*** which are resistant to inactivation by human serum. The retroviral ***vectors*** of the invention are resistant to complement inactivation and are produced from a cell line which is also resistant to lysis by human serum. Cell lines of the invention include the HOS, Mv-1-Lu, HT1080, TE671, and human 293 cell lines, as well as cell lines derived therefrom. To produce said ***vectors*** , a polynucleotide encoding at least the viral envelope protein, but not the entire viral RNA, is utilized. Viruses of the invention include the Moloney Murine Leukemia virus, the feline endogenous virus ***RD114*** , BaEV, SSAV, FeLY-B, NZB virus, avian leukosis virus, and HVJ virus. The invention is also directed to gene therapy employing the provided retroviral ***vectors*** , wherein such ***vectors*** contain at least one polynucleotide encoding a therapeutic agent.
RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 18 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9
AN 1999:238943 BIOSIS
DN PREV199900238943
TI A sodium-dependent neutral-amino-acid transporter mediates infections of feline and baboon endogenous retroviruses and simian type D retroviruses.
AU Tailor, Chetankumar S. (1); Nouri, Ali; Zhao, Yuan; Takeuchi, Yasuhiro; Kabat, David
CS (1) Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Rd., Portland, OR, 97201-3098 USA
SO Journal of Virology, (May, 1999) Vol. 73, No. 5, pp. 4470-4474. ISSN: 0022-538X.
DT Article
LA English
SL English
AB The type D simian retroviruses cause immunosuppression in macaques and have been reported as a presumptive opportunistic infection in a patient with AIDS. Previous evidence based on viral interference has strongly suggested that the type D simian viruses share a common but unknown cell surface receptor with three type C viruses: feline endogenous virus (***RD114***), baboon endogenous virus, and avian reticuloendotheliosis virus. Furthermore, the receptor gene for these viruses has been mapped to human chromosome 19q13.1-13.2. We now report the isolation and characterization of a cell surface receptor for this group of retroviruses by using a human T-lymphocyte cDNA library in a retroviral ***vector*** . Swiss mouse fibroblasts (NIH 3T3), which are naturally resistant to ***RD114*** , were transduced with the retroviral library and then challenged with an ***RD114*** -pseudotyped virus containing a dominant selectable gene for puromycin resistance. Puromycin selection yielded 12 cellular clones that were highly susceptible to a beta-galactosidase-encoding lacZ(***RD114***) pseudotype virus. Using PCR primers specific for ***vector*** sequences, we amplified a common 2.9-kb product from 10 positive clones. Expression of the 2.9-kb cDNA in Chinese hamster ovary cells conferred susceptibility to ***RD114*** , baboon endogenous virus, and the type D simian retroviruses. The 2.9-kb cDNA predicted a protein of 541 amino acids that had 98% identity with the previously cloned human Na+-dependent neutral-amino-acid transporter Bo. Accordingly, expression of the ***RD114*** receptor in NIH 3T3 cells resulted in enhanced cellular uptake of L-(3H)alanine and L-(3H)glutamine. RNA blot (Northern) analysis suggested that the ***RD114*** receptor is widely expressed in human tissues and cell lines, including hematopoietic cells. The human Bo transporter gene has been previously mapped to 19q13.3, which is closely linked to the gene locus of the ***RD114*** receptor.

L3 ANSWER 19 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
AN 1999:202382 BIOSIS
DN PREV199900202382
TI The ***RD114*** /simian type D retrovirus receptor is a neutral amino acid transporter.
AU Rasko, John E. J.; Battini, Jean-Luc; Gottschalk, Rebecca J.; Mazo, Ilya; Miller, A. Dusty (1)
CS (1) Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Room C2-023, Seattle, WA, 98109-1024 USA
SO Proceedings of the National Academy of Sciences of the United States of America, (March 2, 1999) Vol. 96, No. 5, pp. 2129-2134. ISSN: 0027-8424.
DT Article
LA English
AB The ***RD114*** /simian type D retroviruses, which include the feline endogenous retrovirus ***RD114*** , all strains of simian

immunosuppressive type D retroviruses, the avian reticuloendotheliosis group including spleen necrosis virus, and baboon endogenous virus, use a common cell-surface receptor for cell entry. We have used a retroviral cDNA library approach, involving transfer and expression of cDNAs from highly infectable HeLa cells to nonpermissive NIH 3T3 mouse cells, to clone and identify this receptor. The cloned cDNA, denoted RDR, is an allele of the previously cloned neutral amino acid transporter ATB0 (SLC1A5). Both RDR and ATB0 serve as retrovirus receptors and both show specific transport of neutral amino acids. We have localized the receptor by radiation hybrid mapping to a region of about 500-kb pairs on the long arm of human chromosome 19 at q13.3. Infection of cells with ***RD114*** /type D retroviruses results in impaired amino acid transport, suggesting a mechanism for virus toxicity and immunosuppression. The identification and functional characterization of this retrovirus receptor provide insight into the retrovirus life cycle and pathogenesis and will be an important tool for optimization of gene therapy using ***vectors*** derived from ***RD114*** /type D retroviruses.

L3 ANSWER 20 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:46304 BIOSIS
DN PREV200000046304
TI Efficient transduction of CD34+ and CD34+, CD38- human hematopoietic cells with SCID repopulating cell (SRC) potential with an oncoretroviral ***vector*** pseudotyped with a feline endogenous virus (***RD114***) envelope protein.
AU Kelly, Patrick F. (1); Vandergriff, Jody A. (1); Vanin, Elio F. (1); Nienhuis, Arthur W. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 611a. Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology New Orleans, Louisiana, USA December 3-7, 1999 The American Society of Hematology . ISSN: 0006-4971.
DT Conference
LA English

L3 ANSWER 21 OF 32 CAPLUS COPYRIGHT 2002 ACS
AN 1999:475626 CAPLUS
DN 132:54669
TI Enhanced retroviral transduction efficiency of pancreatic tumor cell lines using different envelope glycoproteins
AU Howard, Bradley D.; Boenicke, Lars; Schneider-Brachert, Wulf; Kalthoff, Holger
CS Molecular Oncology Research Laboratory, Clinic for General Surgery, Christian Albrechts University, Kiel, 24105, Germany
SO Ann. N. Y. Acad. Sci. (1999), 880(Cell and Molecular Biology of Pancreatic Carcinoma), 366-370
CODEN: ANYAA9; ISSN: 0077-8923
PB New York Academy of Sciences
DT Journal
LA English
AB The authors tested the possible influence of different media components on the transduction efficiency and gene expression of transduced cells in pancreatic cell lines. The authors used a retroviral ***vector*** contg. the hEGFP gene to demonstrate that pseudotyping retroviral ***vectors*** with VSV-G glycoproteins provided the best transduction efficiency for human pancreatic tumor cells as compared to either MLV-4070A or CEV ***RD114*** pseudotyped retroviral ***vectors*** . The authors also found higher levels of VSV-G transduced pancreatic cells when DMEM plus NEAA was used as a culture medium as compared to RPMI plus NEAA.
RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 22 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:42339 BIOSIS
DN PREV200000042339
TI Efficient gene transfer into canine hematopoietic repopulating cells using ***RD114*** pseudotyped retroviral ***vectors*** .
AU Goerner, M. (1); Storb, R. (1); Rasko, J. E. R. (1); Miller, A. D. (1); Kiem, H. P. (1)
CS (1) Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA USA
SO Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 357a. Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology New Orleans, Louisiana, USA December 3-7, 1999 The American Society of Hematology . ISSN: 0006-4971.
DT Conference
LA English

L3 ANSWER 23 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 11
AN 1998:411486 BIOSIS
DN PREV199800411486
TI CrFK feline kidney cells produce an ***RD114*** -like endogenous virus that can package murine leukemia virus-based ***vectors*** .
AU Baumann, Joerg G.; Guenzburg, Walter H. (1); Salmons, Brian
CS (1) Inst. Virology, Univ. Veterinary Sci., Josef-Baumann-Gasse 1, A-1210 Vienna Austria
SO Journal of Virology, (Sept., 1998) Vol. 72, No. 9, pp. 7685-7687. ISSN: 0022-538X.
DT Article
LA English
AB The feline kidney cell line CrFK is used extensively for viral infectivity assays and for study of the biology of various retroviruses and derived ***vectors*** . We demonstrate the production of an endogenous, ***RD114*** -like, infectious retrovirus from CrFK cells. This virus also is shown to efficiently package Moloney murine leukemia virus ***vectors*** .

L3 ANSWER 24 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12
AN 1998:165530 BIOSIS
DN PREV199800165530
TI Development of improved adenosine deaminase retroviral ***vectors*** .
AU Onodera, Masafumi; Nelson, David M.; Yachie, Akihiro; Jagadeesh, G. Jayashree; Bunnell, Bruce A.; Morgan, Richard A.; Blaese, R. Michael (1)
CS (1) Clinical Gene Therapy Branch, NHGRI, NIH, Build. 10, Room 10C103, 10 Center Dr., MSC 1852, Bethesda, MD 20892-1852 USA
SO Journal of Virology, (March, 1998) Vol. 72, No. 3, pp. 1769-1774. ISSN: 0022-538X.
DT Article
LA English
AB A series of adenosine deaminase (ADA) retroviral ***vectors*** were designed and constructed with the goal of improved performance over the PA317/LASN ***vector*** currently used in clinical trials. First, the bacterial selectable-marker neomycin phosphotransferase (neo) gene was removed to create a "simplified" ***vector*** . Second, the Moloney murine leukemia virus long terminal repeat (LTR) promoter used for ADA expression was replaced with either the myeloproliferative sarcoma virus (MPSV) or SL3-3 LTR. Supernatant from each ADA ***vector*** was used to transduce ADA-deficient (ADA-) B- and T-cell lines as well as primary peripheral blood mononuclear cells (PBMC) from an ADA- severe combined immunodeficiency patient. Total ADA enzyme activity and ADA activity per integrant in the transduced cells demonstrated that the MPSV LTR splicing ***vector*** design provided the highest level of ADA expression per cell. This ADA(MPSV) ***vector*** was then tested in packaging cell lines containing either the gibbon ape leukemia virus envelope (PG13 cells), the murine amphotropic envelope (FLYA13 cells), or the feline endogenous virus ***RD114*** envelope (FLYRD18 cells). The results indicate that FLYRD18/ADA(MPSV), a simplified ADA retroviral ***vector*** with the MPSV LTR, provides a 17-fold-higher level of ADA expression in human lymphohematopoietic cells than the PA317/LASN ***vector*** currently in use.

L3 ANSWER 25 OF 32 CAPLUS COPYRIGHT 2002 ACS
AN 1997:448080 CAPLUS
DN 127:81634
TI Production of retroviral ***vectors*** using herpesvirus ***vectors*** and their use in gene therapy
IN Epstein, Alberto Luis; Cosset, Francois-Loic; Savard, Nathalie
PA Centre National De La Recherche Scientifique, Fr.; Epstein, Alberto Luis; Cosset, Francois-Loic; Savard, Nathalie
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9719182	A1	19970529	WO 1996-FR1817	19961118
W: CA, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
FR 2741358	A1	19970523	FR 1995-13676	19951117
FR 2741358	B1	19980102		
PRAI FR 1995-13676		19951117		

AB A method for producing retroviral ***vectors*** useful for transferring nucleic acid sequences into eukaryotic cells, wherein a eukaryotic cell is infected with at least one herpetic viral ***vector*** , is disclosed. The retroviral elements needed to complete the retroviral cycle are provided by the herpetic ***vector*** (s) alone or in combination with retroviral elements within the genome of the eukaryotic cell. Titers of retroviral ***vectors*** in excess of 108 pfu/mL may be produced with this procedure. This method also permits prodn. of retroviral ***vectors*** in cells normally not infected by a retrovirus. The ***vectors*** may be used in gene therapy for treatment of diseases such as cancer, AIDS, neurodegenerative diseases, etc. Thus, E5 or M64A cells are transfected with pA-HCMV-GPE then superinfected with defective virus HSV-1 D30EBA to produce the herpesvirus ***vector*** pA-HCMV-GPE/D30EBA. (The E5 and M64A cells contain the IE3 gene missing from virus D30EBA while the pA-HCMV-GPE plasmid contains the gag, pol and env genes of Moloney murine leukemia virus.). TE-lac2 cells contg. the retroviral expression cassette LTR-.phi.-LacZ-LTR were infected with the pA-HCMV-GPE/D30EBA ***vector*** and cultured to prep. the retroviral ***vector*** . These retroviral ***vectors*** were capable of infecting 3T3 cells and expressing the LacZ gene.

L3 ANSWER 26 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 13
AN 1997:267414 BIOSIS
DN PREV199799574017
TI Molecular cloning of Mus dunni endogenous virus: An unusual retrovirus in a new marine viral interference group with a wide host range.
AU Bonham, Lynn; Wolgamot, Greg; Miller, A. Dusty (1)
CS (1) Fred Hutchinson Cancer Res. Cent., 1100 Fairview Ave. North, Seattle, WA 98109 USA
SO Journal of Virology, (1997) Vol. 71, No. 6, pp. 4663-4670. ISSN: 0022-538X.
DT Article
LA English
AB Mus dunni endogenous virus (MDEV) is activated from cells of the Asian wild mouse M. dunni (also known as Mus terricolor) in response to treatment with either 5-iodo-2'-deoxyuridine or hydrocortisone. MDEV represents a new murine retrovirus interference group and thus appears to use a different receptor for entry into cells than do other murine retroviruses. Here we show that MDEV is also not in the gibbon ape leukemia virus or ***RD114*** virus interference groups. A retroviral ***vector*** with an MDEV pseudotype was capable of efficiently infecting a wide variety of cells from different species, indicating that the MDEV receptor is widely expressed. We isolated a molecular clone of this virus which exhibited no hybridization to any cloned retrovirus examined, suggesting that MDEV has an unusual genome. One copy of a possible retrovirus element that weakly hybridized with MDEV was present in the genomes of laboratory strains of mice, while no such elements were present in other species examined. A virus activated by 5-iodo-2'-deoxyuridine from cells of a BALB/c mouse, however, was not related to MDEV by either hybridization or interference analyses.

L3 ANSWER 27 OF 32 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 96205519 EMBASE
DN 1996205519
TI Development of amphotropic murine retrovirus ***vectors*** resistant to inactivation by human serum.
AU Pensiero M.N.; Wysocki C.A.; Nader K.; Kikuchi G.E.
CS Genetic Therapy Inc,Gaithersburg, MD 20878, United States
SO Human Gene Therapy, (1996) 7/9 (1095-1101). ISSN: 1043-0342 CODEN: HGTHE3
CY United States
DT Journal; Article
FS 004 Microbiology
022 Human Genetics
LA English

SL English
AB Replication-deficient amphotropic retrovirus ***vectors*** (RV) or RV-producer cells are being developed for a variety of human gene therapy strategies. One of the hurdles to in vivo use of these agents is their inactivation by components of human serum. Murine leukemia viruses (MLV), from which most current RV are derived, are known to be inactivated by human serum via activation of the classical complement cascade. Other type C retroviruses, e.g., ***RD114*** and BaEV, are resistant to inactivation by human serum when derived from infection of human and mink cells but not murine cells. We hypothesized that amphotropic RV could be made resistant to human serum inactivation if a more appropriate producer cell could be found. To test this hypothesis, RV were made using a variety of human (293, HOS, TE671) and murine (NIH-3T3) cell types as the producer cell. The parental cell lines, RV-producer cells, and RV themselves were evaluated for sensitivity to inactivation by human serum. Results showed that the murine MH-3T3 cell line, the NIH-3T3-derived PA317 producer cell line, and RV derived from it were all sensitive to human serum inactivation. In contrast, all human cell lines tested were resistant to lysis. RV and RV-producer cells derived from 293 cells were also resistant; RV derived from HOS cells were resistant. Surprisingly, while TE671 cells were resistant, TE671-derived RV were sensitive to inactivation. To test whether expression of the amphotropic envelope protein was responsible for conferring this serum sensitivity to the RV, env was expressed in the absence of gag and pol in TE671 cells. However, TE671 cells expressing env were resistant to human serum inactivation. These observations have important implications for use of RV and RV-producer cells for human gene therapy.

L3 ANSWER 28 OF 32 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 14
AN 96175705 EMBASE
DN 1998175705
TI Comparison of efficiency of infection of human gene therapy target cells via four different retroviral receptors.
AU Porter C.D.; Collins M.K.L.; Tailor C.S.; Parkar M.H.; Cosset F.-L.; Weiss R.A.; Takeuchi Y.
CS Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road,London SW3 6JB, United Kingdom
SO Human Gene Therapy, (1996) 7/8 (913-919).
ISSN: 1043-0342 CODEN: HGTHE3
CY United States
DT Journal; Article
FS 022 Human Genetics
LA English
SL English
AB The relative efficiency of transduction of gene therapy target cells was measured for retroviruses bearing the envelopes of amphotropic murine leukemia virus (MLV-A), xenotropic murine leukemia virus (MLV-X), gibbon ape leukemia virus (GALV), feline leukemia virus subgroup B (FeLV-B), and the feline endogenous virus ***RD114*** . These viruses use various cell-surface receptors. Activated peripheral blood lymphocytes (PBL) and primary melanoma cultures were infected relatively poorly by MLV-X pseudotypes. ***RD114*** pseudotypes infected PBL relatively well, whereas bone marrow progenitor cells were efficiently infected by all viruses. Helper-free virus bearing the envelopes of MLV-A, ***RD114*** , or GALV was similarly tested. All infected melanoma or bone marrow progenitor cells efficiently, whereas MLV-A was relatively inefficient for infection of PBL. The general utility of ***RD114*** pseudotyped virus for gene delivery coupled with its resistance to inactivation by human serum makes this envelope the most suitable choice for in vivo gene therapy.

L3 ANSWER 29 OF 32 CAPLUS COPYRIGHT 2002 ACS
AN 1995:937745 CAPLUS
DN 124:77712
TI High-titer packaging cells producing recombinant retroviruses resistant to human serum
AU Cosset, Francois-Loic; Takeuchi, Yasuhiro; Battini, Jean-Luc; Weiss, Robin A.; Collins, Mary K. L.
CS Chester Beatty Lab., Inst. Cancer Res., London, SW3 6JB, UK
SO J. Virol. (1995), 69(12), 7430-6
CODEN: JOVIAM; ISSN: 0022-538X
DT Journal
LA English
AB Novel retroviral protein expression constructs were designed to retain minimal retroviral sequences and to express dominant selectable markers by reinitiation of translation after expression of the viral genes. HT1080 cells were selected as producer cells for their ability to release high-titer viruses that are resistant to inactivation by human serum. Two HT1080-based packaging cell lines which produce Moloney murine leukemia virus cores with envelope glycoproteins of either amphotropic murine leukemia virus (FLYA13 line) or cat endogenous virus ***RD114*** (FLYRD18 line) are described. Direct comparison with previous retroviral packaging systems indicated that 100-fold higher titers of helper-free recombinant viruses were released by the FLYA13 and FLYRD18 lines.

L3 ANSWER 30 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 15
AN 1995:34669 BIOSIS
DN PREV199598048969
TI Type C retrovirus inactivation by human complement is determined by both the viral genome and the producer cell.
AU Takeuchi, Yasuhiro; Cosset, Francois-Loi C.; Lachmann, Peter J.; Okada, Hidechika; Weiss, Robin A.; Collins, Mary K. L. (1)
CS (1) Chester Beatty Lab., Inst. Cancer Res., 237 Fulham Rd., London SW3 6JB UK
SO Journal of Virology, (1994) Vol. 68, No. 12, pp. 8001-8007.
ISSN: 0022-538X.
DT Article
LA English
AB The inactivation of type C retroviruses by human serum may be a considerable impediment to the use of retroviral ***vectors*** in vivo for gene therapy. Here we show that virus inactivation is dependent both on the virus and on the cell line used to produce the virus. All viruses produced from murine NTH 3T3 or dog Cf2ThS+L- cells are sensitive to human serum. In contrast, those produced from mink Mv-1-Lu and human HOS or TE671 cells are at least partially resistant, with the exception of murine leukemia viruses. In particular, the feline endogenous virus ***RD114*** is completely resistant to a panel of eight human sera when produced from Mv-1-Lu or HOS cells. This differential resistance is controlled by the viral envelope proteins. Virus inactivation can be correlated with the ability of the producer cells to be lysed by human serum. Inactivation of sensitive viruses requires the classical pathway of complement but does not require virion lysis.

L3 ANSWER 31 OF 32 CAPLUS COPYRIGHT 2002 ACS
AN 1992:505730 CAPLUS
DN 117:105730
TI ***Vectors*** with enhancer and promoter domains of retrovirus or feline RD-114 virus long terminal repeat for gene therapy or technology
IN Roy-Burman, Pradip; Spodick, David A.
PA University of Southern California, USA
SO U.S., 19 pp.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5112767	A	19920512	US 1988-164280	19880304

AB The enhancer and promoter domains of the long terminal repeats (LTRs) of feline endogenous RD-114 proviral loci and exogenous RD-114 provirus are cloned for use in tissue-specific expression of heterologous genes. Also shown was a glycine tRNA primer binding site that is located downstream of the enhancer and promoter domains. ***Vectors*** contg. these enhancer and promoter domains were constructed from the pSVO-CAT contg. a promoterless bacterial CAT reporter gene. These enhancer and promoter domains, EX-LTR and CRL-3, increased levels of expression of the CAT gene compared to the SV40 early promoter-enhancer domain by 10-fold and 3-fold resp.

L3 ANSWER 32 OF 32 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 16
AN 1992:165327 BIOSIS
DN BA93:87652
TI RETROVIRAL PSEUDOTYPES PRODUCED BY RESCUE OF A MOLONEY MURINE LEUKEMIA VIRUS ***VECTOR*** BY C-TYPE BUT NOT D-TYPE RETROVIRUSES.
AU TAKEUCHI Y; SIMPSON G; VILE R G; WEISS R A; COLLINS M K L
CS CHESTER BEATTY LABS., INST. CANCER RES., 237 FULHAM ROAD, LONDON SW3 6JB, UK.
SO VIROLOGY, (1992) 186 (2), 792-794.
CODEN: VIRLAX. ISSN: 0042-6822.
FS BA; OLD
LA English
AB Human HOS cells containing a Moloney murine leukemia virus (Mo-MLV) recombinant genome were infected by a panel of retroviruses. The C-type viruses simian sarcoma associated virus, feline leukemia virus subgroup B, and the feline endogenous virus ***RD114*** were able to form pseudotypes with the Mo-MLV genome, which transferred a selectable marker gene to target cells; however, Human T cell leukemia virus-1 and the D-type viruses Mason-Pfizer monkey virus and simian retrovirus-1 failed to rescue the Mo-MLV ***vector*** . Further characterization of the ***RD114*** pseudotype demonstrated that it retained the receptor specificity of ***RD114*** and will therefore prove useful in receptor characterization.

=> FIL STNGUIDE

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=> FIL BIOSIS EMBASE CAPLUS
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CA SUBSCRIBER PRICE	0.00	-4.34

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FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 12:10:51 ON 31 JAN 2002
L1 244 S RD114
L2 61 S L1 AND VECTOR?
L3 32 DUP REM L2 (29 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 12:14:39 ON 31 JAN 2002

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 12:47:19 ON 31 JAN 2002

=> s retronectin
L4 23 RETRONECTIN

=> dup rem l4

PROCESSING COMPLETED FOR L4
L5 18 DUP REM L4 (5 DUPLICATES REMOVED)

=> d bib abs l5

L5 ANSWER 1 OF 18 CAPLUS COPYRIGHT 2002 ACS
AN 2001:676635 CAPLUS
DN 135:236393
TI Highly efficient gene transfer into human repopulating stem cells by RD114 envelope protein pseudotyped retroviral vector particles which pre-adsorb on ***retronectin*** -coated plates
IN Kelly, Patrick F.; Vanin, Elio F.
PA St. Jude Children's Research Hospital, USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001066150	A2	20010913	WO 2001-US7212	20010307

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2001051375	A1	20011213	US 2001-801302	20010307
PRAI US 2000-187534	P	20000307		

AB The present invention relates to a method for efficiently introducing exogenous genes into stem cells, particularly human stem cells. The method optionally includes the steps of inducing the proliferation of target cells by pre-stimulation with cytokines and/or growth factors, followed by incubating these cells with RD114-pseudotyped vector particles. In a specific embodiment, the vector particles are ***retronectin*** -immobilized or ultracentrifugation-concd. retroviral vector particles pseudotyped with the feline endogenous retrovirus (RD114) envelope protein. The present invention further discloses a method for somatic gene therapy, which can be used for various therapeutic applications and involves introducing a gene of interest contained within the retroviral genome into human repopulating stem cells followed by introducing these cells into a human host. Finally, the present invention discloses a method for monitoring the efficiency of the stem cell-mediated gene transfer based on detecting the presence of the genes (or the expression products) of the retroviral vector in various stem cell-derived lineages of the host.

=> d bib abs 2-y
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L5 ANSWER 2 OF 18 CAPLUS COPYRIGHT 2002 ACS
AN 2001:168163 CAPLUS
DN 134:203423
TI Improved transduction of pluripotent hematopoietic stem cells using retroviral gene delivery system, and use of retroviral particles in treatment of various disorders
IN Verstegen, Monique Maria Andrea; Wognum, Albertus Wernerus; Wagemaker, Gerard
PA Erasmus Universiteit Rotterdam, Neth.
SO PCT Int. Appl., 28 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001016341	A1	20010308	WO 2000-NL611	20000901

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1081227	A1	20010307	EP 1999-202859	19990902

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRAI EP 1999-202859	A	19990902
EP 1999-203875	A	19991119

AB The invention provides the materials and methods for improved transduction of CD34+ cells, from bone marrow or umbilical cord blood (UCB), using gene delivery vehicles of retroviral origin (retroviral particles). The invention relates that the CD34+ cells are cultured in the presence of fibronectin or ***retronectin*** . The invention also provides for use of transduced CD34+ cells in the expression of a heterologous protein when introduced into mammalian hosts. The invention further provides a method for prodn. of said gene delivery vehicles (retroviral particles). Finally, the invention provides: (1) pharmaceutical compns. comprising said retroviral particles, and (2) use of said compns. in treatment of a hereditary disorder or a pathol. condition related to a genetic aberration, and/or in prepn. of medicament for treatment of various disorders. The invention discussed that useful nucleic acid mols. can be provided to stem cells using the material and methods provided. The invention also discussed that an important variable in the efficiency of transduction is the ratio between the no. of cells and no. of transducing particles. The invention utilized the above improved method to transduce CD34+ human UCB cells, and human and rhesus monkey bone marrow cells with a retrovirus carrying the EGFP (enhanced green fluorescent protein) gene. The transduced cells were then transplanted into irradiated mice or rhesus monkeys and the expression of EGFP in bone marrow was detd.
RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:264678 BIOSIS
DN PREV200100264678
TI Cancer immunotherapy by genetically engineered effector lymphocytes redirected by chimeric receptors.
AU Eshhar, Zelig (1); Pinthus, Jehonathan H. (1); Waks, Tova (1); Bendavid, Alain (1); Schindler, Daniel G. (1)
CS (1) Weizmann Institute of Science, Rehovot, 76100 Israel
SO FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A1200. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
DT Conference
LA English
SL English
AB To expand the recognition spectrum of effector lymphocytes and redirect them to predefined targets, notably cancer cells, we endowed T and NK cells with antibody-type specificity, using chimeric receptor genes. Several configurations of chimeric receptors have been designed, mostly employing the anti-tumor antibody V region in the form of single chain variable fragment (scFv) as the recognition domain. As another recognition unit, we have replaced the extracellular scFv with the Neuregulin/NDF ligand, which binds to human adenocarcinoma cells over-expressing members of the erb-B onco-receptor family. To avoid anergy and antigen induced cell death, we have included the co-stimulatory CD28 molecule as part of the chimeric receptor and found that such a tri-partite receptor, containing scFv linked to CD28 as spacer and co-stimulatory moiety and the FcR g as stimulatory domain can indeed serve to fully activate resting T cells of transgenic mice harboring such chimeric receptor. To determine and optimize the clinical applicability of the chimeric receptor approach we have used an efficient procedure for the transduction of CD3/CD28 activated human T cells, employing retrovectors expressing GaLV envelopes and ***RetroNectin*** , a routine expression the chimeric receptors can be achieved in 40-70% of the cells. As a model, we have established a few human prostate cancer xenografts in SCID mice and demonstrated that local administration of human T cells expressing an HER/2-specific chimeric receptor could cause a complete rection of the tumors. We believe that prostate cancer is an excellent candidate for the chimeric receptor gene-immunotherapy not only because direct, intratumoral application of the genetically engineered lymphocytes is possible and because the metastatic pattern of prostate tumor (bones, lymph nodes) is readily accessible to T cells, but also because 'biological prostatectomy' is acceptable.

L5 ANSWER 4 OF 18 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 1
AN 2001240395 EMBASE
TI The impact of ex vivo cytokine stimulation on engraftment of primitive hematopoietic cells in a non-human primate model.
AU Dunbar C.E.; Takatoku M.; Donahue R.E.; Sharkis S.J.; Broxmeyer H.E.; Storb R.F.; Eaves C.J.; Moore M.A.S.
CS Dr. C.E. Dunbar, Molecular Hematopoiesis Section, NHLBI, NIH, 9000 Rockville Pike, Bethesda, MD 20892, United States. dunbarc@nhlbi.nih.gov
SO Annals of the New York Academy of Sciences, (2001) 938/- (236-245).
Refs: 20
ISSN: 0077-8923 CODEN: ANYAA
CY United States
DT Journal; Conference Article
FS 025 Hematology
026 Immunology, Serology and Transplantation
LA English
SL English
AB The impairment of engraftment ability after ex vivo or in vivo stimulation of hematopoietic stem cells, potentially related to induction of active cell cycling, has recently been a topic of intense interest. Our group has used the non-human primate autologous transplantation model and genetic marking to investigate a number of questions in hematopoiesis with direct relevance to human clinical applications. The issue of a potential reversible engraftment defect would have many implications for gene therapy and allogeneic or autologous transplantation. Initial in vitro studies with rhesus CD34(+) cells indicated that after 4 days of stimulatory culture in stem cell factor (SCF), megakaryocyte growth and development factor (MDGF), and flt3 ligand (FLT), transfer of the cells to SCF alone on ***retronectin*** (FN) support resulted in decreased active cycling and a halt to proliferation, without a loss of viability or induction of apoptosis. We then directly compared the engraftment potential of cytokine-stimulated cells versus those transferred to SCF on FN alone before reinfusion. SCF/G-CSF mobilized CD34(+) cells from three animals were split into two parts and transduced with either of two retroviral marking vectors for 4 days in the presence of SCF/FLT/MGDF on FN. One aliquot was cryopreserved, and the other was continued in culture without transduction for 2 days in the presence of SCF alone on FN. After total body irradiation, both aliquots were thawed and reinfused into each animal. In all animals, the level of marking from the fraction continued in culture for 2 days with SCF on FN was significantly higher than the level of marking from the aliquot transduced for 4 days without the 2-day period in SCF alone. This approach may allow more efficient engraftment of successfully transduced or ex vivo expanded cells by avoiding active cell cycling at the time of reinfusion.

L5 ANSWER 5 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2000:294355 BIOSIS
DN PREV200000294355
TI Efficient transduction of human hematopoietic repopulating cells generating stable engraftment of transgene-expressing cells in NOD/SCID mice.
AU Barquinero, Jordi; Segovia, Jose Carlos; Ramirez, Manuel; Limon, Ana; Guenechea, Guillermo; Puig, Teresa; Briones, Javier; Garcia, Juan; Bueren, Juan Antonio (1)
CS (1) Department of Molecular and Cellular Biology, CIEMAT, Madrid Spain
SO Blood, (May 15, 2000) Vol. 95, No. 10, pp. 3085-3093. print.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB In an attempt to develop efficient procedures of human hematopoietic gene

therapy, retrovirally transduced CD34+ cord blood cells were transplanted into NOD/SCID mice to evaluate the repopulating potential of transduced grafts. Samples were prestimulated on ***Retronectin*** -coated dishes and infected with gibbon ape leukemia virus (GALV)-pseudotyped FMEV vectors encoding the enhanced green fluorescent protein (EGFP). Periodic analyses of bone marrow (BM) from transplanted recipients revealed a sustained engraftment of human hematopoietic cells expressing the EGFP transgene. On average, 33.6% of human CD45+ cells expressed the transgene 90 to120 days after transplantation. Moreover, 11.9% of total NOD/SCID BM consisted of human CD45+ cells expressing the EGFP transgene at this time. The transplantation of purified EGFP+ cells increased the proportion of CD45+ cells positive for EGFP expression to 57.7% at 90 to 120 days after transplantation. At this time, 18.9% and 4.3% of NOD/SCID BM consisted of CD45+/EGFP+ and CD34+/EGFP+ cells, respectively. Interestingly, the transplantation of EGFP- cells purified at 24 hours after infection also generated a significant engraftment of CD45+/EGFP+ and CD34+/EGFP+ cells, suggesting that a number of transduced repopulating cells did not express the transgene at that time. Molecular analysis of NOD/SCID BM confirmed the high levels of engraftment of human transduced cells deduced from FACS analysis. Finally, the analysis of the provirus insertion sites by conventional Southern blotting indicated that the human hematopoiesis in the NOD/SCID BM was predominantly oligoclonal.

L5 ANSWER 8 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:317226 BIOSIS
DN PREV200100317226
TI Storage of factor VIII (FVIII) in the alpha-granules of human platelets following retroviral transduction and transplantation of human CD34+ cells into NOD-SCID mice.
AU Wilcox, David A. (1); Rosenberg, Jonathan B.; Johnson, Bryon D. (1); Montgomery, Robert R. (1)
CS (1) Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 803a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB In order to develop methods for gene therapy of disorders affecting hemostasis, we transduced Isolex(R) selected CD34+ cells (Nexell Therapeutics) from human mobilized peripheral blood with a retroviral vector encoding human FVIII (Chiron Technologies). CD34+ cells were transduced on plates coated with ***RetroNectin*** (Takara Shuzo) in the presence of SCF, flt-3/flk-2 ligand, IL-6, and pegylated recombinant human Megakaryocyte Growth and Differentiation Factor (Kirin Brewery). Indirect immunofluorescence analysis using antibodies against human FVIII, vWF, and the megakaryocyte-specific marker, glycoproteins (GP) IIb-IIIa revealed that megakaryocytes derived from transduced CD34+ cells in vitro could synthesize FVIII and traffick it to alpha-granules in association with von Willebrand factor (vWF). This result was similar to trafficking previously observed for these molecules to Weibel-Palade bodies in FVIII-transduced endothelial cells. FVIII was also detected in the cytoplasm of cultured cells that were negative for vWF or GPIIb-IIIa staining, indicating that transduction was not limited to the megakaryocyte lineage. To examine the effect of FVIII expression in platelets, in vivo, FVIII-transduced CD34+ cells were transplanted into NOD-SCID mice treated with a sublethal dose (350 cGy) of irradiation. Flow cytometric analysis using antibodies specific for human GPIIb-IIIa revealed that circulating human platelets comprised up to 40% of the total platelet population in whole blood isolated from the mice during 2-6 weeks post-transplant. Immunofluorescence analysis using confocal microscopy revealed a punctuate staining for FVIII that was colocalized with vWF to alpha-granules in a subpopulation of human platelets isolated from murine whole blood. In contrast, FVIII was not detected in murine platelets. These results indicate that human megakaryocytes can synthesize and store FVIII with vWF in alpha-granules that can be retained in progeny platelets. We speculate that FVIII could undergo regulated release from platelets following physiologic hemostatic response to vessel injury. This raises the possibility of developing a locally inducible secretory pool of FVIII in platelets of patients with hemophilia A following autologous transplantation of FVIII-transduced CD34+ peripheral blood cells.

L5 ANSWER 7 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322415 BIOSIS
DN PREV200100322415
TI Ex vivo expansion of primitive hematopoietic cells by reduction of p21cip1/waf1 expression level.
AU Stier, S. (1); Cheng, T. (1); Miura, N. (1); Dombkowski, D. (1); Sarmento, L. M. (1); Scadden, D. T. (1)
CS (1) Exp. Hematology, Massachusetts General Hospital, Charlestown, MA USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 867a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The quiescence of hematopoietic stem cells is critical to prevent the exhaustion of the hematopoietic system in vivo, while limiting the clinical applicability of ex vivo stem cell expansion and gene therapy. Current protocols for ex vivo expansion of stem cells involve the use of differentiation inducing cytokines, which often leads to a decreased multipotentiality of the expanded cell pool. Implicated in the maintenance of stem cell quiescence is the CDK inhibitor p21cip1/waf1 (p21) (Science 287,2000:1804). p21-knock out mice showed an increase of absolute hematopoietic stem cell number under normal homeostatic conditions and premature death due to hematopoietic cell depletion after cell cycle specific myelotoxic injury in comparison to wildtype mice. These findings suggest an alternative strategy of ex vivo stem cell expansion maintaining the multipotentiality of stem cells by altering the p21 expression levels. Therefore, we transduced CD34+ and CD34+38- cord blood cells with a VSV-G pseudotyped lentiviral vector containing full length p21-antisense (p21-AS). After transduction for 20 hrs on two successive days in the presence of KL(50ng/ml), Flt-3-L(50ng/ml), TPO(25ng/ml), IL-3(10ng/ml) and polybrene(4mug/ml) on ***retronectin*** coated wells a transduction efficiency of 45-55% for the control vector and 25-35% for the p21-AS vector could be observed. The p21-AS transduced CD34+ and CD34+38- cells showed a 3.4- and 2.7-fold increase in the CFU-mix colony number in comparison to the control vector transduced cells (CD34+: 9.3 vs. 2.7 col. per 600 cells, p=0.016; CD34+38-: 19.2 vs. 7.1 col. per 600 cells, p=0.013), whereas the total colony number was not significantly increased. The stem cell number present in the transduced cell population was directly measured by limit-dilution LTC-IC assays. A significant increase in primitive cells in the p21-AS transduced CD34+ and CD34+38- cells in comparison to the control vector transduced cells was noted (CD34+: 33.5 vs. 19.3 LTC-ICs per 105 cells, p=0.037; CD34+38-: 205.6 vs. 82.4 LTC-ICs per 105 cells). Furthermore, 8 weeks after transplantation into sublethal irradiated NOD/SCID mice p21-AS transduced CD34+ cells showed a 20-fold higher repopulating potential than control vector tranduced cells. These results demonstrate a specific expansion of primitive cells in hematopoietic cell pools by reduction of p21 expression. Therefore, reducing p21 expression level offers a new approach for ex vivo hematopoietic stem cell expansion.

L5 ANSWER 8 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322183 BIOSIS
DN PREV200100322183
TI Comparative analysis of gene marking and lineage development in SCID-repopulating cells derived from cord blood or mobilized peripheral blood.
AU Pollok, Karen E.; van der Loo, Johannes C. M.; Cooper, Ryan J.; Hartwell, Jennifer R.; Miles, Katherine R.; Breese, Robert; Williams, David A.
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 589a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB Efficient transfer and expression of therapeutic genes in long-term repopulating cells derived from G-CSF-mobilized peripheral blood CD34+ cells (MPB) is a priority for many clinical gene therapy protocols. The efficiency of gene transfer in MPB SCID-repopulating cells (SRCs) was compared to gene transfer in SRCs derived umbilical cord blood CD34+ cells (CB). Pre-stimulated CB or MPB cells were infected twice on FN CH-296 (***Retronectin*** (R), Takara Shuzo) utilizing a GALV-pseudotyped MFG-EGFP retroviral vector at an identical multiplicity of infection (MOI = 2) and transplanted into NOD/SCID mice. Flow cytometric analysis and clonogenic assays indicated that approximately 70% of the input CB cells were EGFP+, while 35-50% of input MPB cells were EGFP+. This discrepancy was even more striking in SRCs derived from CB versus those derived from MPB. At 6-9 weeks post-transplant, 35-40% of the CB-derived human cells repopulating NOD/SCID mice in bone marrow (BM) and spleen (n=11) were EGFP+, while in MPB transplant recipients, human cells in BM and spleen were only 0.4-4.0% EGFP+ (n=23). Low levels of gene marking in MPB were confirmed by PCR of individual human colonies from the BM. In recipients of both CB and MPB, immature B-cell progenitors (CD34+, CD19+), mature B cells (CD34-, CD19+) and myeloid (CD45+, CD33+) lineages contained gene-marked cells. SRCs in MPB may require a longer pre-stimulation time for entry into cell cycle. Therefore, MPB (n=41) was transduced after 4-8 days of pre-stimulation. Although human cell engraftment was observed under all pre-stimulation conditions, gene-transfer levels in both lymphoid and myeloid lineages ranged from 0.5-8.0% for MPB. An exception was noted in one MPB donor in which gene transfer following a 6-day pre-stimulation period resulted in 6-16% EGFP+ human cells in the BM. PKH2 staining of MPB was employed to evaluate proliferation following pre-stimulation. After 6-8 days of ex vivo expansion followed by transduction, approximately 1-2.0% of the MPB was PKH2+, EGFP- indicative of a small population of cells that was still refractive to stimulation and transduction (n=5). Long-term repopulating cells still existed in MPB ex vivo expanded for up to 10 days, since human cells were detected by genomic Southern in the bone marrow of secondary NOD/SCID transplants. In conclusion, a significant discrepancy exists in the ability to effectively introduce genes into SRCs derived from MPB as compared to CB. Strategies utilizing in vivo selection or alternative vector systems may be necessary to achieve high levels of transduced MPB SRCs.

L5 ANSWER 9 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302193 BIOSIS
DN PREV200100302193
TI Multilineage transduction of non-human primate CD34+ hematopoietic cells using RD-114 pseudotyped oncoretroviruses.
AU Kelly, Patrick F. (1); Bonifacino, Aylin C.; Carrington, Jody A. (1); Agricola, Brian A.; Metzger, Mark E.; Kluge, Kim A.; Nienhuis, Arthur W. (1); Donahue, Robert E.; Vanin, Elio F. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 525a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The relative quiescence of the hematopoietic stem cell (HSC) and the low level of viral receptor expression are known to contribute to the low efficiency of retroviral gene transfer into HSCs of large animals and humans. We have previously reported that RD114-pseudotyped retroviruses could efficiently transduce cord blood CD34+ cells after 24-48 hours pre-stimulation and a single exposure to the viral particles preloaded onto ***RetroNectin*** -coated plates. Based on these results we evaluated gene transfer of RD114-pseudotyped murine retroviruses using non-human primate CD34+ peripheral blood (PB) cells in the rhesus autologous transplant model. SCF/G-CSF-mobilized rhesus monkey PB were collected and enriched for CD34+ cells. These cells were cultured in serum-containing medium with high concentrations of SCF, FLT-3 and IL-6 and exposed to RD114-pseudotyped particles preloaded onto ***RetroNectin*** -coated plates at 48 hours and 72 hours. After 96 hours in culture, cells were harvested and infused into irradiated recipients (2 X 500 cGy, n=5). The transduction efficiency of the infused cells was 35-55% based on EGFP expression. In all animals we have observed multilineage engraftment with persistence of EGFP expression after 6-8 weeks post-transplantation, a result that was not achieved with a similar construct pseudotyped with the amphotropic envelope protein. In the first animal transplanted, we observed high levels of multilineage engraftment of EGFP+ cells (as high as 98% in granulocytes) over the first 20 weeks post transplantation. After 26 weeks multilineage expression has stabilized at 8-10%. Serial genomic southern analysis for both proviral

integrity and integration site indicated that vector silencing was not occurring and that the engraftment of gene modified cells was oligoclonal. The second recipient displayed similar kinetics but died from transplant related complications 8 weeks post-transplantation. Subsequent animals have achieved lower levels of EGFP expression (1-3%) suggesting that transduction conditions using this pseudotype remains to be optimized. These results suggest oncoretroviral vectors pseudotyped with the RD114 envelope protein could be useful for achieving clinically relevant levels of gene transfer into human pluripotent hematopoietic cells.

L5 ANSWER 10 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302190 BIOSIS
DN PREV200100302190
TI *In vivo expansion of gene-modified hematopoietic cells by the selective amplifier gene in a nonhuman primate model.*
AU *Hanazono, Yutaka (1); Nagashima, Takeyuki; Shibata, Hiroaki; Ageyama, Naohide; Asano, Takayuki (1); Ueda, Yasuji; Kume, Akihiro (1); Terao, Keiji; Hasegawa, Mamoru; Ozawa, Keiya (1)*
CS (1) Div. Genet. Therapeut., Jichi Med. Sch., Tochigi Japan
SO *Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 524a. print.* Meeting Info.: 42nd Annual Meeting of the American Society of Hematology *San Francisco, California, USA December 01-05, 2000 American Society of Hematology* . *ISSN: 0006-4971.*
DT Conference
LA *English*
SL English
AB *Although hematopoietic stem cells (HSCs) have been pursued as desirable targets for gene therapy, clinical studies indicate that the gene transfer efficiency into human HSCs is too low to be of clinical utility in most situations. To overcome this problem, we developed a method of in vivo expansion of transduced cells. In this system, target cells are harnessed with the selective amplifier gene (SAG), a chimeric gene of the G-CSF receptor and the estrogen receptor hormone-binding domain. We deleted the G-CSF-binding domain from the chimeric gene to abolish the responsiveness to G-CSF and introduced a mutation (Y703F) to prevent the differentiation signal transduction. We demonstrated that the SAG product predominantly transmits the proliferation signal with the minimal differentiation signal in response to estrogen in vitro. We then examined the in vivo effect of the SAG in a cynomolgus macaque model. Cynomolgus bone marrow CD34+ cells were transduced with MSCV-based, GALV-pseudotyped retroviral vectors with or without the SAG (n=3). The supernatant transduction was performed for 4 days with* ***retronectin*** *(supplied by Takara) and cytokines including Flt-3 ligand. The transduced cells were reinfused into each myeloablated monkey (500cGy X 2). After transplantation, bone marrow cells were taken and each colony formed by the cells was subjected to PCR in search of the provirus. In two monkeys without the SAG, around 10% of colony-forming progenitors contained the provirus for 1 year posttransplant. In the other monkey (female) with the SAG, although only 10% of progenitors contained the provirus before reinfusion, the provirus was detected in approximately 40% of progenitors posttransplant even without administration of estrogen. Some progenitors with the SAG responded to the endogenous estrogen. Since the proportion of the provirus-containing progenitors dropped to 5% 6 months posttransplant, estradiol was administered to the monkey. The progenitors with the provirus then increased to 30% in response to the exogenous estrogen. These results suggest that, with inclusion of the SAG in retroviral vectors, gene modified hematopoietic progenitors could be selectively expanded in vivo by treatment with estrogen.*

L5 ANSWER 11 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322016 BIOSIS
DN PREV200100322016
TI *Comparison of three retroviral envelopes for high efficiency gene transfer into human marrow mesenchymal cells.*
AU *Hofmann, Ted J. (1); Capizzani, Tony R. (1); Kelly, Patrick F. (1); Vanin, Elio F. (1); Horwitz, Edwin M. (1)*
CS *(1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA*
SO *Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 220a. print.* Meeting Info.: 42nd Annual Meeting of the American Society of Hematology *San Francisco, California, USA December 01-05, 2000 American Society of Hematology* . *ISSN: 0006-4971.*
DT Article; Conference
LA *English*
SL English
AB *Bone marrow stromal cell (MSCs) are marrow mesenchymal cells that are ideal vehicles for delivery of therapeutic proteins in gene therapy protocols. A major obstacle to any successful gene therapy strategy is obtaining high efficiency transduction of the target cells. To optimize transduction of MSCs for clinical trials, we compared the effect of the retroviral envelope on gene transfer efficiency. Three different pseudotypes of a murine stem cell viral vector, encoding the green fluorescent protein (GFP) as a marker, were produced: amphotropic (Ampho) in PA317 cells, GALV in PG13 cells, and RD114 (RD) in FLYRD18 cells. The titer of each supernatant was determined using HeLa cells: Ampho = 4.1 X 104, GALV1 = 3.4 X 103, GALV2 = 1.2 X 105, and RD = 5.0 X 105 tu/ml. Following a standard 3-day transduction protocol, the human MSCs were analyzed by flow cytometry to determine the percentage of GFP positive cells. First, MSCs were transduced with Ampho (MOI = 0.2) yielding 92%; GALV1 (MOI = 0.02), 46%; GALV2 (MOI = 0.6), 68%; and RD (MOI = 2.5), 86% gene transfer. Next, MSCs were transduced with RD at an MOI of 0.2 (equivalent to Ampho) and 83% gene transfer was observed, not significantly different from the 86% transduction obtained using undiluted RD or the 92% obtained with Ampho. Finally, MSCs were transduced with either Ampho or RD at an MOI of 0.02 (equivalent to GALV1). Ampho transduced 77% and RD 61% of the MSCs, compared to 46% for GALV1. Notably, dilute RD (61%) and dilute Ampho (77%) transduced MSCs as well as the higher titer GALV2 (68%). Northern blot analysis showed an unexpected ratio (8:4:1) for the mRNAs of RDR (RD114 receptor), Pit-1 (GALV receptor), and Pit-2 (amphotropic receptor). Although RD and Ampho have similar potential to mediate gene transfer into MSCs, the mRNA for RDR is 8-fold more abundant than Pit-2 mRNA. Further, Pit-1 is 4-fold more abundant than Pit-2 despite the apparent lower gene transfer efficiency. We then compared the standard transduction of MSCs to transduction using* ***RetroNectin*** *coated dishes and found no difference in gene transfer efficiency. We conclude that amphotropic and RD114 pseudotyped vectors are more effective for mediating gene transfer into MSCs. Further, more abundant receptor mRNA does not necessarily indicate a greater potential for transduction by the respective viral pseudotype. A higher titer GALV pseudotyped vector may be adequate for efficient transduction but sufficiently high titer PG13 supernatant has been difficult to generate. Additionally,* ***RetroNectin*** *does not enhance gene transfer in our system. Thus, RD114 or amphotropic envelopes are preferred for clinical trials of MSC gene therapy.*

L5 ANSWER 12 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322004 BIOSIS
DN PREV200100322004
TI *Highly efficient retroviral gene transfer to human cord blood CD34+/CD38low and NOD/SCID repopulating cells using a simplified transduction protocol.*
AU Relander, Thomas (1); Karlsson, Stefan (1); Richter, Johan (1)
CS *(1) Molecular Medicine and Gene Therapy, University Hospital, Lund Sweden*
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 217a. print. *Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology* . ISSN: 0006-4971.
DT *Article; Conference*
LA English
SL *English*
AB We investigated retroviral gene transfer to human cord blood CD34+/CD38+, *CD34+/CD38low and NOD/SCID repopulating cells and compared transduction efficiency using an MSCV based vector with the gene for GFP (MGIN) which was packaged into 3 different cell lines: PG13 (GALV), 293GPG (VSV-G) or GP+env-AM12 (amphotropic). Viral titer was 1-3X106 inf. units/ml for PG13-MGIN and AM12-MGIN; for 293GPG-MGIN up to 107. Cord blood CD34+ cells were sorted into CD38 low (6% lowest) or CD38+ fractions to study kinetics of transduction and were cultured in serum-free medium with MGDF, FL and SCF (100 ng/ml) before transduction with a single 24 hour hit in* ***Retronectin*** *(RN) coated wells preloaded with vector on days 0-5. Efficient transduction of CD38+ cells was observed already after one day of pre-stimulation and then was at approximately the same level through day 4; 59-67% (PG13), 23-30% (293GPG) and 39-51 % (AM12) However, CD38low cells were not efficiently transduced until day 3 day but level of GFP+ cells was then approximately the same as for the CD38+ cells; 62%, 29 % and 39 %, respectively. In 3 NOD/SCID experiments, cells were cultured as above for 48 hrs before transduction (with serum (SC) or serum free (SF)) on RN pre-loaded with virus alone followed by addition of 1/10 volume of virus supernatant at 72 hrs without further manipulations. At 96 hrs cells were harvested and injected into irradiated NOD/SCID mice (250.000 EE/mouse), which were analyzed at 6 w. Compared to engraftment of fresh cells (44% SD 25,6) transduction under SC but not SF conditions resulted in significantly lower engraftment. All three envelopes tested efficiently transduced SRC but transduction measured by FACS and GFP+ CFU was significantly higher for PG13SF when compared to 293GPG and AM12. Transplantation of fresh and PG13SF transduced cells at limiting dilution showed no loss of engraftment capacity of transduced cells. Engraftment of GFP positive human cells with as low as 15.625EE was observed. Conclusions: Highly efficient retroviral transduction of primitive human hematopoietic progenitors without loss of repopulating activity can be achieved using a very simple protocol with RN preloaded with virus. The PG13 pseudotyped vector used under serum free conditions gave the best results.*

L5 ANSWER 13 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:321993 BIOSIS
DN PREV200100321993
TI *Fetal liver stromal cell line AFT024 enhances gene transfer in primitive human hematopoietic cells in mobilized peripheral blood.*
AU *Van Der Loo, Johannes C. M. (1); Eaton, Kristin S. (1)*
CS (1) Medicine, University of Minnesota, Minneapolis, MN USA
SO *Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 215a. print.* Meeting Info.: 42nd Annual Meeting of the American Society of Hematology *San Francisco, California, USA December 01-05, 2000 American Society of Hematology* . *ISSN: 0006-4971.*
DT Article; Conference
LA *English*
SL English
AB *NOD/SCID transplant studies show that primitive hematopoietic cells in human G-CSF mobilized peripheral blood (MPB) are more difficult to transduce than cells from umbilical cord blood (UCB). We hypothesize that primitive hematopoietic cells in MPB are refractive to gene transfer (GT) due to insufficient cytokine stimulation prior to retroviral infection. Earlier studies have demonstrated a positive effect of the fetal liver stromal cell line AFT024 on the maintenance of primitive hematopoietic cells ex vivo in the presence of low doses of early acting cytokines. Based on these data we propose that AFT024 may enhance the level of GT in primitive hematopoietic cells in MPB. To test this hypothesis, CD34+ cells from MBP were cultured for four days in the presence or absence of irradiated AFT024 cells using trans-well (non-contact) cultures with either G-CSF, SCF and TPO (GST; 100 ng/mL) or Flt3-L, SCF, IL-7 and TPO (FS7T; 10-20 ng/mL), followed by infection with a GALV-pseudotyped MFG-EGFP retroviral vector on* ***Retronectin*** *(R) (Takara Shuzo) on two consecutive days (m.o.i. ≈ 2). The level of GT as well as the level of expansion was quantified using CFC and LTC-IC assays. AFT024 had a positive effect on the expansion of both CFC and LTC-IC (both 2-fold increase) independent of the cytokines used. In the presence of AFT024, the level of GT in CFC (ranging from 1 to 26% in BFU-E and CFU-GM; n = 10) was higher in the groups pre-stimulated with GST, while the level of GT in LTC-IC (ranging from 1.5 to 47%) was higher with FS7T (n=6). Overall, the recovery of transduced LTC-IC was 5 to 6-fold higher in the presence of AFT024 with FS7T as compared to our previously used strategy using GST in the absence of stroma (p<0.001). As the expansion and, therefore, the cell cycle behavior of CFC and LTC-IC was similar, these data indicate that the growth factors used have an additional but differential effect on the level of GT in primitive and less primitive cells. Finally, for application in clinical protocols, we demonstrate that AFT024 cells can be replaced by AFT024-conditioned medium without loss of transduced LTC-IC (n=5, ns). In conclusion, we demonstrate that the recovery of transduced primitive hematopoietic cells in G-CSF MPB can be enhanced using low doses of early acting cytokines and (a) soluble factor(s) produced by the cell line AFT024.*

L5 ANSWER 14 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:321986 BIOSIS
DN PREV200100321986
TI *Lentiviral vectors effectively transfer and express human glucose 6-phosphate dehydrogenase (G6PD) in primitive human hematopoietic cells (HSC) engrafting NOD/SCID mice.*
AU Notaro, Rosario (1); Levy, Carolyn Fein (1); De Angioletti, Maria (1);

Vanegas, Olga Camacho (1); Rovira, Ana (1); Sadelain, Michel (1); Luzatto, Lucio (1)
CS (1) Human Genetics, MSKCC, New York, NY USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 213a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB Lentiviral (LV) vectors, based on HIV, are emerging as powerful tools for transducing HSC. However, comparative data on LV vectors versus conventional murine leukemia virus (MLV) vectors with respect to optimizing transduction conditions and measuring transduction efficiency have been scarce. We have previously, transferred and expressed hG6PD in bona fide HSC using MLV vectors pseudotyped with the vesicular stomatitis virus G glycoprotein (VSVG). We have now constructed a VSVG-pseudotyped LV vector in which the hG6PD cDNA is under the transcriptional control of the CMV promoter. This LV vector was used to transduce lineage negative cord blood cells in serum-free medium (MOI apprx25) on ***retronectin*** coated plates. We tested various transduction conditions: (1) 5 hrs with or without cytokines; (2) 12 hrs of pre-culture followed by one or more transduction cycles of 12 hrs with cytokines. The transduced cells were (a) plated for hematopoietic colony forming cells (CFC) and (b) injected into sub-lethally irradiated NOD/SCID mice. In most of the expressing CFC the level of the transferred G6PD was at least as much as that of the endogenous G6PD. The LV vector was able to transfer and express 6GPD in a significant proportion of committed progenitors under all transduction conditions. However, in order to obtain expression in primitive HSC, 12 hours of pre-culture time and the use of cytokines were needed. In conclusion, primitive human HSC that are able to engraft into NOD/SCID mice need "priming" to be effectively transduced by LV vectors; transduction efficiency with LV vectors (apprx40%) is higher than that we have previously obtained with MLV vectors (apprx20%) using a MOI of apprx100. A definitive comparison between LV and MLV vectors under identical transduction conditions is needed.

L5 ANSWER 15 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:385012 BIOSIS
DN PREV200000385012
TI Centrifugation-enhanced retroviral gene transduction of human CD34+ cells in RetroNectinTM-coated gas permeable X-FoldTM containers.
AU Thornton, J. (1); Goel, A.; Tseng-Law, J.; Szalay, P.; Malech, H.; Van Epps, D.; Freimark, B.
CS (1) Nexell Therapeutics Inc., Irvine, CA USA
SO Experimental Hematology (Charlottesville), (July, 2000) Vol. 28, No. 7 Supplement 1, pp. 125. print.
Meeting Info.: 29th Annual Meeting of the International Society for Experimental Hematology Tampa, Florida, USA July 08-11, 2000 International Society for Experimental Hematology
. ISSN: 0301-472X.
DT Conference
LA English
SL English

L5 ANSWER 16 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 1999:397479 BIOSIS
DN PREV199900397479
TI Optimization of retroviral gene transduction of mobilized primitive hematopoietic progenitors by using thrombopoietin, Flt3, and Kit ligands and ***RetroNectin*** culture.
AU Murray, Lesley (1); Luens, Karin; Tushinski, Robert; Jin, Liang; Burton, Michelle; Chen, Jingyi; Forestell, Sean; Hill, Beth
CS (1) SyStemix, 3155 Porter Drive, Palo Alto, CA, 94304 USA
SO Human Gene Therapy, (July 20, 1999) Vol. 10, No. 11, pp. 1743-1752.
ISSN: 1043-0342.
DT Article
LA English
SL English
AB We have investigated the ability of several cytokine combinations to improve retrovirus-mediated transduction of human primitive hematopoietic progenitors (PHPs) from mobilized peripheral blood (MPB). Retroviral infection of CD34+ cells was performed by culture on fibronectin fragment CH-296 (***RetroNectin*** , RN), using the truncated human nerve growth factor receptor (NGFR) as the transgene reporter. Transgene expression among progeny of PHPs was assayed by FACS analysis after long-term stromal culture (LTC). Transgene delivery to PHPs was assessed by PCR of individual stromal culture-derived methylcellulose colonies (LTC-CFCs). Compared with interleukin 3 (IL-3), IL-6, and leukemia inhibitory factor (LIF), the combination of thrombopoietin (TPO), Flt3 ligand (FL), and Kit ligand (KL) effected a 73-fold increase in NGFR expression among CD34+ cells (to 14%) and a 14-fold increase in NGFR expression among total cells (to 10%) after LTC. In addition, a 2.4-fold increase in neo gene marking of LTC-CFCs was observed. A preclinical study comparing the effect of high-speed centrifugation ("spinoculation") or culture on RN during exposure to retroviral particles in teflon cell culture bags showed no difference in the efficiency of transduction of PHPs between these two methods.

L5 ANSWER 17 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:46648 BIOSIS
DN PREV200000046648
TI Immobilization of suspension cells on extracellular matrix: An on and off affair.
AU Prokopishyn, Nicole L. (1); Barron, Gina L. (1); Carsrud, N. D. Victor (1); Brown, David B. (1); Yannariello-Brown, Judith (1)
CS (1) Gene-Cell, Inc., Houston, TX USA
SO Blood, (Nov. 15) Vol. 94, No. 10 SUPPL. 1 PART 2, pp. 187b.
Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology New Orleans, Louisiana, USA December 3-7, 1999 The American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English

L5 ANSWER 18 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:113886 BIOSIS
DN PREV199900113886
TI Transduction kinetics of non-human primate immuno-selected CD34+ cells using retroviral and lentiviral vectors that express the green fluorescent protein.
AU Donahue, R. E. (1); Rowe, T. K.; Sorrentino, B. P.; Hawley, R. G.; An, D. S.; Chen, I. S. Y.; Wersto, R. P.
CS (1) Hematol. Branch, NHLBI, Rockville, MD USA
SO Blood, (Nov. 15, 1998) Vol. 92, No. 10 SUPPL. 1 PART 1-2, pp. 376B.
Meeting Info.: 40th Annual Meeting of the American Society of Hematology Miami Beach, Florida, USA December 4-8, 1998 The American Society of Heamatology
. ISSN: 0006-4971.
DT Conference
LA English

=> s trasduct? and stem cell? and FLT3
2 FILES SEARCHED...
L6 0 TRASDUCT? AND STEM CELL? AND FLT3

=> s transduct? and stem cell? and FLT3
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L7 0 TRANSDUCT? AND STEM CELL? AND FLT3

=> s transduc? and stem cell? and FLT3
2 FILES SEARCHED...
L8 0 TRANSDUC? AND STEM CELL? AND FLT3

=> s transduc? and hematopoie? and FLT3
L9 0 TRANSDUC? AND HEMATOPOIE? AND FLT3

=> s transduc? and hematopoie? and FLT3
L10 220 TRANSDUC? AND HEMATOPOIE? AND FLT3

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L11 145 DUP REM L10 (75 DUPLICATES REMOVED)

=> s l11 and py<2000
1 FILES SEARCHED...
L12 80 L11 AND PY<2000

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L12 ANSWER 1 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:133630 BIOSIS
DN PREV200000133630
TI Retroviral gene transfer into human ***hematopoietic*** cells: An in vitro kinetic study.
AU Briones, Javier; Puig, Teresa; Limon, Ana; Petriz, Jordi; Garcia, Joan; Barquinero, Jordi (1)
CS (1) Department of Cryobiology and Cell Therapy, Institut de Recerca Oncologica, Gran Via km 2.7, L'Hospitalet, Barcelona, 08907 Spain
SO Haematologica., (***June, 1999***) Vol. 84, No. 6, pp. 483-488.
ISSN: 0390-6078.
DT Article
LA English
SL English
AB Background and Objective. Successful gene therapy applications require optimized strategies to increase gene transfer efficiency into hematopoietic progenitor cells (HPCs) with long-term repopulating ability. One of the issues that needs to be clarified is how ***hematopoietic*** cells proliferate, differentiate and express the transgene after each cycle of ***transduction*** . We investigated the kinetics of cell expansion, CD34 antigen expression and ***transduction*** efficiency of human ***hematopoietic*** cells in culture conditions commonly used in retroviral gene transfer protocols. Design and Methods. Purified CD34+ cells from cord blood (n=5) or leukapheresis products (n=9) and a retroviral vector encoding an enhanced version of the green fluorescent protein (EGFP) were used. Target cells were exposed daily to vector-containing supernatants and a combination of interleukin 3 (IL-3), interleukin 6 (IL-6), stem cell factor (SCF) and ***Flt3*** -ligand (FL). Cell samples were harvested from the cultures and analyzed at 24 hour intervals for seven consecutive days. Results. We found that CD34+ cells proliferated and differentiated under our culture conditions. The number of genetically modified cells increased after each cycle of ***transduction*** . Median numbers of cells positive for both CD34 and EGFP increased steadily over the culture period, but after day four most of the EGFP+ cells had a low CD34 expression. Interpretation and Conclusions. Culturing and ***transducing*** CD34+ cells for longer periods of time under these conditions might be detrimental for ex vivo gene transfer applications since the ***transduced*** cells are likely to have a decreased potential for long-term engraftment and repopulation in vivo.

L12 ANSWER 2 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:59438 BIOSIS
DN PREV200000059438
TI Gp130-Signaling synergizes with FL and TPO for the long-term expansion of cord blood progenitors.
AU Rappold, I. (1); Watt, S. M.; Kusadasi, N.; Rose-John, S.; Hatzfeld, J.; Ploemacher, R. E.
CS (1) MRC Molecular Haematology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DS UK
SO Leukemia (Basingstoke), (***Dec., 1999***) Vol. 13, No. 12, pp. 2036-2048.
ISSN: 0887-6924.
DT Article
LA English
SL English
AB We investigated the effect of a new fusion protein of IL-6 and the soluble IL-6R, H-IL-6, on the long-term ex vivo expansion of ***hematopoietic*** progenitors derived from AC133+ cord blood cells. H-IL-6, which acts on both IL-6Ralpha-positive and IL-6Ralpha-negative cells, effectively synergized with FL and TPO with or without SCF for the propagation of primitive progenitors. However, IL-6 showed a greater synergistic effect with FL and TPO than H-IL-6 for long-term progenitor propagation. During the first 6 weeks of culture under stroma-free serum-containing conditions, IL-6 induced a 1.96 +- 0.64-fold higher expansion of nucleated cells, a 2.28 +- 0.33-fold higher expansion of CD34+ cells and a 2.74 +- 0.28-fold higher expansion of CD34+ AC133+ cells than H-IL-6 in combination with FL and TPO. The propagation of week 6 CAFC was up to four-fold higher in the presence of IL-6 than with H-IL-6. While the expansion of CD34+ and CD34+ AC133+ cells dropped after 5-7 weeks in the stroma-free cultures with FL, TPO and H-IL-6, a sustained expansion for 12

weeks was obtained in the presence of FL, TPO and IL-6. Stroma-contact greatly enhanced the progenitor expansion induced by FL and TPO or FL, TPO and H-IL-6 although the highest proliferation was again obtained in the presence of IL-6. In contrast, the presence of SCF resulted in increased differentiation. Since the majority of primitive progenitors are proposed to be IL-6Ralpha-negative, the results suggest that the synergistic effect of IL-6 is mediated by accessory cells, which have been more effectively stimulated by IL-6 than by the fusion peptide, H-IL-6, in this culture system.

L12 ANSWER 3 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:28175 BIOSIS
DN PREV200000028175
TI Optimization of retroviral-mediated gene transfer to human NOD/SCID mouse repopulating cord blood cells through a systematic analysis of protocol variables.
AU Hennemann, Burkhard; Conneally, Eibhlin; Pawliuk, Robert; Leboulch, Philippe; Rose-John, Stefan; Reid, Dianne; Chuo, Jean Y.; Humphries, R. Keith; Eaves, Connie J. (1)
CS (1) Terry Fox Laboratory, 601 West 10th Avenue, Vancouver, BC, V5Z 1L3 Canada
SO Experimental Hematology (Charlottesville), (***May, 1999***) Vol. 27, No. 5, pp. 817-825.
ISSN: 0301-472X.
DT Article
LA English
SL English
AB Retroviral ***transduction*** of human ***hematopoietic*** stem cells is still limited by lack of information about conditions that will maximize stem cell self-renewal divisions in vitro. To address this, we first compared the kinetics of entry into division of single human CD34+CD38- cord blood (CB) cells exposed in vitro to three different ***flt3*** -ligand (FL)-containing cytokine combinations. Of the three combinations tested, FL + hyper-interleukin 6 (HIL-6) yielded the least clones and these developed at a slow rate. With either FL + Steel factor (SF) + HIL-6 + thrombopoietin (TPO) or FL + SF + interleukin 3 (IL-3) + IL-6 + granulocyte-colony-stimulating factor (G-CSF), >90% of the cells that formed clones within 6 days undertook their first division within 4 days, although not until after 24 hours. These latter two, more stimulatory, cytokine combinations then were used to assess the effect of duration of cytokine exposure on the efficiency of ***transducing*** primitive CB cells with a gibbon apeleukemia virus-pseudotyped murine retroviral vector containing the enhanced green fluorescent protein (GFP) cDNA and the neomycin resistance gene. Fresh lin- CB cells exposed once to medium containing this virus plus cytokines on fibronectin-coated dishes yielded 23% GFP+ CD34+ cells and 52-57% G418-resistant CFC when assessed after 2 days. Prestimulation of the target cells (before exposing them to virus) with either the four or five cytokine combination increased their susceptibility. In both cases, the effect of prestimulation assessed using the same infection protocol was maximal with 2 days of prestimulation and resulted in 47-54% GFP+ CD34+ cells and 67-69% G418-resistant CFC. Repeated daily addition of new virus (up to three times), with assessment of the cells 2 days after the last addition of fresh virus, gave only a marginal improvement in the proportion of ***transduced*** CD34+ cells and CFC, but greatly increased the proportion of ***transduced*** LTC-IC (from 40% to >99%). Transplantation of lin- CB cells ***transduced*** using this latter 6-day protocol into NOD/SCID mice yielded readily detectable GFP+ cells in 10 of 11 mice that were engrafted with human cells. The proportion of the regenerated human cells that were GFP+ ranged from 0.2-72% in individual mice and included both human lymphoid and myeloid cells in all cases. High-level reconstitution with ***transduced*** human cells was confirmed by Southern blot analysis. These findings demonstrate that transplantable ***hematopoietic*** stem cells in human CB can be reproducibly ***transduced*** at high efficiency using a 6-day period of culture in a retrovirus-containing medium with either FL + SF + HIL-6 + TPO or FL + SF + IL-3 + IL-6 + G-CSF in which virus is added on the third, fourth, and fifth day.

L12 ANSWER 4 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:23915 BIOSIS
DN PREV200000023915
TI Thrombopoietin, ***flt3*** , and kit ligands together suppress apoptosis of human mobilized CD34+ cells and recruit primitive CD34+Thy-1+ cells into rapid division.
AU Murray, Lesley J. (1); Young, Judy C.; Osborne, Linda J.; Luens, Karin M.; Scollay, Roland; Hill, Beth L.
CS (1) SyStemix, 3155 Porter Drive, Palo Alto, CA, 94304 USA
SO Experimental Hematology (Charlottesville), (***June, 1999***) Vol. 27, No. 6, pp. 1019-1028.
ISSN: 0301-472X.
DT Article
LA English
SL English
AB Various combinations of cytokines have profoundly different effects on inhibition of apoptosis and stimulation of self-renewal division of ***hematopoietic*** stem cells (HSC) in short-term, ex vivo culture. Our goal was to quantitate expansion of cells with a primitive CD34+Thy-1+ phenotype, as well as cell cycling, division history, differentiation, and apoptosis of CD34+ cells enriched from normal donor mobilized peripheral blood (MPB) cells. The balance of these parameters determines the net number of transplantable HSC produced in ex vivo cultures. Comparing several different combinations of cytokines added to 90-hour cultures of MPB CD34+ cells, thrombopoietin (TPO), ***flt3*** ligand (FL), and c-kit ligand (KL) gave the best result, with the lowest percentage of apoptotic cells and a mean 1.2-fold increase in the number of CD34+Thy-1+ cells. A combination of interleukin 3 (IL-3), interleukin 6 (IL-6), and leukemia inhibitory factor (LIF) gave the worst outcome, including a decrease of CD34+Thy-1+ cell number to a mean of 30% of the starting cell number. Cell division history was tracked using the dye 5-(and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE). Division of CD34+Thy-1+ cells was faster and more synchronous in TPO, FL, and KL than in IL-3, IL-6, and LIF, which left a significant proportion of CD34+ cells undivided. Such detailed analyses of short-term, ex vivo cultures generated "replication scores," which allowed prediction of a sixfold improvement of the efficiency of gene ***transduction*** of primitive ***hematopoietic*** progenitors from MPB, using TPO, FL, and KL to replace IL-3, IL-6, and LIF. Analysis of retroviral ***transduction*** efficiency confirmed the increase of transgene expression from MPB primitive ***hematopoietic*** progenitors assayed after stromal culture was fivefold, validating the usefulness of multiparameter analysis of short-term cultures for survival and replication of CD34+Thy-1+ cells.

L12 ANSWER 5 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:23913 BIOSIS
DN PREV200000023913
TI CD34+ cells from mobilized peripheral blood retain fetal bone marrow repopulating capacity within the Thy-1+ subset following cell division ex vivo.
AU Young, Judy C. (1); Lin, Karen; Hansteen, Gun; Travis, Marilyn; Murray, Lesley J.; Jaing, Li; Scollay, Roland; Hill, Beth L.
CS (1) 3155 Porter Drive, Palo Alto, CA, 94304 USA
SO Experimental Hematology (Charlottesville), (***June, 1999***) Vol. 27, No. 6, pp. 994-1003.
ISSN: 0301-472X.
DT Article
LA English
SL English
AB Ex vivo cell cycling of ***hematopoietic*** stem cells (HSC), a subset of primitive ***hematopoietic*** progenitors (PHP) with engrafting capacity, is required for ***transduction*** with retroviral vectors and to increase transplantable HSC numbers. However, induction of division of HSC ex vivo also may lead to differentiation and loss of in vivo marrow repopulating potential. We evaluated mobilized peripheral blood (MPB) PHP for maintenance of stem cell function after ex vivo culture under conditions that we show can induce cycling of a majority of PHP with minimal differentiation. The following methods were combined: cell labeling with the division tracking dye carboxyfluorescein-diacetate succinimidylester (CFSE), analysis of primitive cell surface marker expression, an ex vivo PHP assay, and an in vivo marrow repopulating assay. MPB-purified CD34+Thy-1+ cells were labeled with CFSE dye and cultured for 112 hours in serum-deprived medium in the presence of the cytokine combinations of thrombopoiet in (TPO), ***flt3*** ligand (FL), and c-kit ligand (KL), or TPO, FL, and interleukin 6 (IL-6). Both cytokine combinations supported division of greater than 95% of cells within 112 hours with an average 2.1-fold (TPO, FL, KL) or 1.3-fold (TPO, FL, IL-6) increase in total cell numbers. An average of 21.6% (TPO, FL, KL) and 27.4% (TPO, FL, IL-6) of the divided cells still expressed the Thy-1 marker after 112 hours. Functional assays were performed to compare cultured and uncultured cells. CD34+Thy-1+CFSElo (post division) cells showed maintenance of cobblestone area-forming cell (CAFC) frequency (a mean of 1/9.0) relative to the starting population of uncultured CD34+Thy-1+ cells (a mean of 1/8.4). In contrast, CD34+ cells that had lost Thy-1 expression during culture (CD34+Thy-1-CFSElo) showed a mean 5.8-fold reduction in CAFC frequency (a mean of 1/52.5). Only the Thy-1-expressing fraction of cells post culture could engraft in vivo in the SCID-hu bone assay. Because the majority of HSC functional activity post culture was found in the CD34+Thy-1+ fraction, we focused on this fraction for subsequent analysis. CFSE labeling allows segregation and purification by flow cytometry of cells having undergone discrete numbers of divisions during culture. Very few cells that divided more than four times in culture still expressed Thy-1. Cells that retained expression of Thy-1 during culture retained CAFC activity relative to fresh CD34+Thy-1+ cells, after undergoing at least two divisions. CAFC frequency decreased after four divisions in culture with TPO, FL, and KL or after three divisions in TPO, FL, and IL-6. We then compared populations of Thy-1+ cells that had undergone sequential numbers of divisions in culture for their ability to engraft in the SCID-hu bone assay. Engrafting ability was retained throughout four divisions in both cytokine combinations. These data demonstrate that primitive MPB CD34+ cells maintain HSC function coincident with Thy-1 expressi on while undergoing two to four divisions under these culture conditions. Essentially all CD34+ Thy-1+ cells divided under the conditions tested, promoting susceptibility to retroviral ***transduction*** .

L12 ANSWER 8 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:507889 BIOSIS
DN PREV199900507889
TI Efficient and durable gene marking of ***hematopoietic*** progenitor cells in nonhuman primates after nonablative conditioning.
AU Rosenzweig, M.; MacVittie, T. J.; Harper, D.; Hempel, D.; Glickman, R. L.; Johnson, R. P.; Farese, A. M.; Whiting-Theobald, N.; Linton, G. F.; Yamasaki, G.; Jordan, C. T.; Malech, H. L. (1)
CS (1) Laboratory of Host Defenses, NIAID, 10 Center Dr, Bldg 10 Room 11N113, MSC 1886, Bethesda, MD, 20892-1886 USA
SO Blood, (***Oct. 1, 1999***) Vol. 94, No. 7, pp. 2271-2286.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB Optimization of mobilization, harvest, and ***transduction*** of ***hematopoietic*** stem cells is critical to successful stem cell gene therapy. We evaluated the utility of a novel protocol involving ***Flt3*** -ligand (***Flt3*** -L) and granulocyte colony-stimulating factor (G-CSF) mobilization of peripheral blood stem cells and retrovirus ***transduction*** using ***hematopoietic*** growth factors to introduce a reporter gene, murine CD24 (mCD24), into ***hematopoietic*** stem cells in nonhuman primates. Rhesus macaques were treated with ***Flt3*** -L (200 mug/kg) and G-CSF (20 mug/kg) for 7 days and autologous CD34+ peripheral blood stem cells harvested by leukapheresis. CD34+ cells were ***transduced*** with an MFGS-based retrovirus vector encoding mCD24 using 4 daily ***transductions*** with centrifugations in the presence of ***Flt3*** -L (100 ng/mL), human stem cell factor (50 ng/mL), and PIXY321 (50 ng/mL) in serum-free medium. An important and novel feature of this study is that enhanced in vivo engraftment of ***transduced*** stem cells was achieved by conditioning the animals with a low-morbidity regimen of sublethal irradiation (320 to 400 cGy) on the day of transplantation. Engraftment was monitored sequentially in the bone marrow and blood using both multiparameter flow cytometry and semi-quantitative DNA polymerase chain reaction (PCR). Our data show successful and persistent engraftment of ***transduced*** primitive progenitors capable of giving rise to marked cells of multiple ***hematopoietic*** lineages, including granulocytes, monocytes, and B and T lymphocytes. At 4 to 6 weeks posttransplantation, 47% +- 32% (n = 4) of granulocytes expressed mCD24 antigen at the cell surface. Peak in vivo levels of genetically modified peripheral blood lymphocytes approached 35% +- 22% (n = 4) as assessed both by flow cytometry and PCR 6 to 10 weeks posttransplantation. In addition, naive (CD45RA+ and CD62L+) CD4+ and CD8+ cells were the predominant phenotype of the marked CD3+ T cells detected at early time points. A high level of markingpersisted at between 10% and 15% of peripheral blood leukocytes for 4 months and at lower levels past 6 months in some animals. A cytotoxic T-lymphocyte response against mCD24 was detected in only 1 animal. This degree of persistent long-lived, high-level gene marking of multiple ***hematopoietic*** lineages, including naive T cells, using a nonablative marrow conditioning regimen

represents an important step toward the ultimate goal of high-level permanent ***transduced*** gene expression in stem cells.

L12 ANSWER 7 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:397479 BIOSIS
DN PREV199900397479
TI Optimization of retroviral gene ***transduction*** of mobilized primitive ***hematopoietic*** progenitors by using thrombopoietin, ***Flt3*** , and Kit ligands and RetroNectin culture.
AU Murray, Lesley (1); Luens, Karin; Tushinski, Robert; Jin, Liang; Burton, Michelle; Chen, Jingyi; Forestell, Sean; Hill, Beth
CS (1) SyStemix, 3155 Porter Drive, Palo Alto, CA, 94304 USA
SO Human Gene Therapy, (***July 20, 1999***) Vol. 10, No. 11, pp. 1743-1752.
ISSN: 1043-0342.
DT Article
LA English
SL English
AB We have investigated the ability of several cytokine combinations to improve retrovirus-mediated ***transduction*** of human primitive ***hematopoietic*** progenitors (PHPs) from mobilized peripheral blood (MPB). Retroviral infection of CD34+ cells was performed by culture on fibronectin fragment CH-296 (RetroNectin, RN), using the truncated human nerve growth factor receptor (NGFR) as the transgene reporter. Transgene expression among progeny of PHPs was assayed by FACS analysis after long-term stromal culture (LTC). Transgene delivery to PHPs was assessed by PCR of individual stromal culture-derived methylcellulose colonies (LTC-CFCs). Compared with interleukin 3 (IL-3), IL-6, and leukemia inhibitory factor (LIF), the combination of thrombopoietin (TPO), ***Flt3*** ligand (FL), and Kit ligand (KL) effected a 73-fold increase in NGFR expression among CD34+ cells (to 14%) and a 14-fold increase in NGFR expression among total cells (to 10%) after LTC. In addition, a 2.4-fold increase in neo gene marking of LTC-CFCs was observed. A preclinical study comparing the effect of high-speed centrifugation ("spinoculation") or culture on RN during exposure to retroviral particles in teflon cell culture bags showed no difference in the efficiency of ***transduction*** of PHPs between these two methods.

L12 ANSWER 8 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:338988 BIOSIS
DN PREV199900338988
TI Soluble bone marrow stroma factors improve the efficiency of retroviral transfer of the human multidrug resistance 1 gene to human mobilized peripheral blood progenitor cells.
AU Schiedlmeier, B.; Buss, E. C.; Veldwijk, M. R.; Zeller, W. J.; Fruehauf, S. (1)
CS (1) Department of Internal Medicine V, University of Heidelberg, Hospitalstr. 3, 69115, Heidelberg Germany
SO Human Gene Therapy, (***June 10, 1999***) Vol. 10, No. 9, pp. 1443-1452.
ISSN: 1043-0342.
DT Article
LA English
SL English
AB ***Hematopoietic*** stem cells (HSCs) are a potential target for the retrovirus-mediated transfer of chemotherapeutic drug resistance genes. For integration of the proviral DNA in the HSC genome cell division is required. In the bone marrow (BM) ***hematopoiesis*** occurs in the vicinity of stroma cells. Soluble stroma components were shown to play a permissive role for the proliferation of lineage-committed and primitive ***hematopoietic*** progenitors in conjunction with cytokines. We investigated the effect of stroma-conditioned medium (SCM) of the FBMD1 cell line on the gene transfer rate of the human multidrug resistance 1 (MDR1) gene contained in the retroviral SF-MDR vector into human mobilized peripheral blood progenitor cells (PBPCs) from tumor patients (n = 14) during transwell ***transduction*** in the presence of the recombinant fibronectin fragment CH-296. Addition of SCM during ***transduction*** increased the gene transfer efficiency into myeloid lineage-committed colony-forming cells by an averageof 1.5-fold (p = 0.02) as detected by an SF-MDR provirus-specific polymerase chain reaction (PCR). These data were paralleled by significantly (p = 0.04 to p = 0.007) higher proportions of MDR1-expressing myelo-monocytic progeny after ***transduction*** in SCM plus interleukin 3 (IL-3), IL-3/ ***Flt3*** ligand (FL), IL-3/IL-6/FL, or IL-3/IL-6/stem cell factor (SCF) when compared with ***transductions*** without SCM as measured by rhodamine-123 exclusion. A similar trend was observed for SCM employed in combination with IL-3/IL-6/SCF/FL or FL/thrombopoietin (TPO)/SCF during ***transduction*** . The latter combination plus SCM yielded the highest proportion, 19.16 +- 3.10% Rh-123dull cells. The beneficial effect of SCM on ***transduction*** efficiency was confirmed in additional four patients' samples, using a serum-free viral supernatant ***transduction*** protocol. As soluble BM stroma factors are able to increase the efficiency of retrovirus-mediated gene transfer into committed progenitor cells, beyond that achieved with fibronectin fragment CH-296, their effect on gene transfer into primitive repopulating ***hematopoietic*** cells may also prove beneficial.

L12 ANSWER 9 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:261846 BIOSIS
DN PREV199900261846
TI Efficient detection and selection of immature rhesus monkey and human CD34+ ***hematopoietic*** cells expressing the enhanced green fluorescent protein (EGFP.
AU Bierhuizen, M. F. A.; Westerman, Y.; Hartong, S. C. C.; Visser, T. P.; Wognum, A. W.; Wagemaker, G. (1)
CS (1) Institute of Hematology, Erasmus University Rotterdam, Dr Molewaterplein 50, 3015 GE, Rotterdam Netherlands
SO Leukemia (Basingstoke), (***April, 1999***) Vol. 13, No. 4, pp. 605-613.
ISSN: 0887-6924.
DT Article
LA English
SL English
AB The feasibility of using the enhanced green fluorescent protein (EGFP) as a selectable reporter molecule of retroviral-mediated gene transfer in immature rhesus monkey and human CD34+ ***hematopoietic*** cells was examined. Retroviral ***transduction*** with the MFG-EGFP retroviral vector resulted in readily detectable EGFP expression in 27% of human and 11-35% of rhesus monkey bone marrow cells, and in 17-38% of rhesus monkey peripheral blood cells mobilized with ***FLT3*** ligand (FL) and granulocyte colony-stimulating factor (G-CSF). In addition, we used the human CD34+ KG1A cell line as a model to study viability and growth of successfully ***transduced*** cells. Cultures of mock- and EGFP-***transduced*** KG1A cells generated equal viable cell numbers for at least 1 month, indicating the absence of a cytotoxic effect of EGFP expression in these cells. FACS selection on the basis of EGFP and CD34 expression resulted in enriched subsets (gtoreq87%) of CD34+ EGFP-negative and CD34+ EGFP-positive KG1A, rhesus monkey and human bone marrow cells, demonstrating the potential of obtaining almost pure populations of ***transduced*** immature ***hematopoietic*** cells. EGFP expression was also readily demonstrated in erythroid and granulocyte/macrophage colonies derived from the CD34+ EGFP-positive rhesus monkey and human bone marrow cells by either inverted fluorescence microscopy or flow cytometry. Using four-color flow cytometry, EGFP expression could also be demonstrated in viable and phenotypically defined immature subpopulations of the CD34+ cells, ie those expressing little or no HLA-DR (rhesus monkey) or CD38 (human) antigens at the cell surface. These results demonstrate that EGFP is a very useful marker to monitor gene transfer efficiency in phenotypically defined immature rhesus monkey and human ***hematopoietic*** cell types and to select for these cells by multicolor flow cytometry prior to transplantation.

L12 ANSWER 10 OF 80 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:204635 BIOSIS
DN PREV199900204635
TI ***Flt3*** signaling involves tyrosyl-phosphorylation of SHP-2 and SHIP and their association with Grb2 and Shc in Baf3/ ***Flt3*** cells.
AU Zhang, Shuli; Mantel, Charlie; Broxmeyer, Hal E. (1)
CS (1) Department of Microbiology/Immunology and the Walther Oncology Center, Indiana University School of Medicine, 1044 West Walnut Street, Building R4, Room 302, Indianapolis, IN, 46202-5254 USA
SO Journal of Leukocyte Biology, (***March, 1999***) Vol. 65, No. 3, pp. 372-380.
ISSN: 0741-5400.
DT Article
LA English
SL English
AB ***Flt3*** ligand (FL) is an early-acting potent co-stimulatory cytokine that regulates proliferation and differentiation of a number of blood cell lineages. Its receptor ***Flt3*** /Flk2 belongs to class III receptor tyrosine kinases that also include the receptors for colony-stimulating factor 1, Steel factor, and platelet-derived growth factor. Using CSF-1 receptor/ ***Flt3*** chimeras, two groups have characterized some of the post-receptor signaling events and substrate specificity of murine ***Flt3*** receptor. However, there are few studies on the signaling pathway through human ***Flt3*** . We examined human ***Flt3*** signaling pathways in a murine IL-3-dependent ***hematopoietic*** cell line Baf3, which stably expresses full-length human ***Flt3*** receptor. This subline proliferates in response to human FL. Like the chimeric murine ***Flt3*** , human ***Flt3*** undergoes autophosphorylation, associates with Grb2, and leads to tyrosine phosphorylation of Shc on ligand binding. We found that SHP-2, but not SHP-1, is tyrosine-phosphorylated by FL stimulation. SHP-2 does not associate with ***Flt3*** , but binds directly to Grb2. SHIP is also tyrosine-phosphorylated and associates with Shc after FL simulation. We further examined the downstream signaling pathway. FL transiently activates MAP kinase. This activation could be blocked by PD98059, a specific MEK inhibitor. PD98059 also blocked cell proliferation in response to FL. These results demonstrate that SHP-2 and SHIP are important components in the human ***Flt3*** signaling pathway and suggest that SHP-2 and SHIP, by forming complexes with adapter proteins Grb2 and Shc, may modulate MAP kinase activation, which may be necessary for the mitogenic signaling of ***Flt3*** .

=> d bib abs 50-55

L12 ANSWER 50 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1999:764177 CAPLUS
DN 132:19626
TI Efficient gene delivery by multiply attenuated HIV-1-based lentiviral ***transducing*** vectors that show efficient packaging
IN Chang, Lung-Ji; Cui, Yan; Iwakuma, Tomoo
PA University of Florida, USA
SO PCT Int. Appl., 197 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 5

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9961598	A2	19991202	WO 1999-US11634	19990526 <--
WO 9961598	A3	20000413		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9942078	A1	19991213	AU 1999-42078	19990526 <--
PRAI US 1998-86635	P	19980526		
WO 1999-US11634	W	19990526		

AB A method of constructing HIV-1-based lentiviral ***transducing*** vectors with increased packaging efficiency and minimal recombination potentials for target gene delivery in gene therapy was described. The parental packaging vector pHP-1 contained a modified 5' HIV-1 LTR, a novel major splice donor site derived from RSV, the entire gag-, pol-env, vif, vpr, vpu, tat, rev genes, and a selectable gpt marker gene, and an SV40 polyadenylation signal and multiple derivs. were generated by deletion and mutation. Deletion in the env, and in the 5' LTR, of vpr, vif, and vpu in these derivs. packaging vectors did not affect the packaging efficiency and these viral particles showed similar protein level and even higher titers compared to the wild type HIV-1 expressing vector. However, tat-minus derivs. are deficient in GAG-POL processing and can be complemented by cotransfecting the packaging cell lines with a tetracycline-inducible construct expressing HIV-1 tat. Two families of ***transducing*** vectors were constructed with pTV.phi. using synthetic packaging signals and pTV.DELTA. using deleted HIV-1 packaging signals in which pTV.phi. were packaged much less efficiently than pTV.DELTA.. These packaging and ***transducing*** vectors efficiently ***transduced*** actively dividing including rhabdomyosarcoma cell TE671, kidney carcinoma

cell 293T, hepatoma cell HepG2 and Hela cells. They also efficiently ***transduced*** non-dividing and terminally differentiated cells including mitomycin C-treated TE671 cell and Hela cell, CD34+ human ***hematopoietic*** stem cell (HSC), primary neurons, monocyte-derived macrophages and mouse leg muscles by i.m. injection. The protocol for HSC ***transduction*** were optimized by coculturing target cells with retroviral producer cells, treating target cells with mitomycin C and cotransfecting the target cells with constructs expressing growth factor such as human IL-3, or G-CSF, or ***flt3*** ligand. HIV-1 essential elements U3, SD, gag AUG, gag-pol, env, tat, rev, and 3' SA sites and all the necessary genes in ***transducing*** vectors were also deletable to minimize the recombination potential and improve the safety of gene therapy. The primary packaging signal were narrowed down into the sequences of SL2 and SL4 by further reducing the overlapped sequences between ***transducing*** vectors and the packaging vectors. The effective gene delivery using these lentiviral vectors has a great potential in human gene therapy.

L12 ANSWER 51 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1999:655742 CAPLUS
DN 132:163968
TI SHC and SHIP phosphorylation and interaction in response to activation of the ***FLT3*** receptor
AU Marchetto, S.; Fournier, E.; Beslu, N.; Aurran-Schleinitz, T.; Dubreuil, P.; Borg, J.-P.; Birnbaum, D.; Rosnet, O.
CS Laboratoire d'Oncologie Moleculaire, Institut Paoli-Calmettes, Marseille, 13288, Fr.
SO Leukemia (***1999***), 13(9), 1374-1382
CODEN: LEUKED; ISSN: 0887-6924
PB Stockton Press
DT Journal
LA English
AB The ***FLT3*** receptor tyrosine kinase and its ligand, FL, regulate the development of ***hematopoietic*** stem cells and early B lymphoid progenitors. FL has a strong capacity to boost prodn. of dendritic and natural killer cells in vivo, thereby providing a new and promising tool for anti-cancer immunotherapy. Intracellular ***FLT3*** signaling involves tyrosine phosphorylation of several cytoplasmic proteins including SHC. We have found that upon ***FLT3*** activation SHC phosphorylation occurs at tyrosine 239/240 and 313. SHC possesses two phosphotyrosine-binding domains: an amino-terminal phosphotyrosine binding (PTB) and a carboxy-terminal Src Homol. 2 (SH2) domain. Neither is required for SHC phosphorylation, but the PTB domain is necessary and sufficient for SHC binding to the SH2 contg. inositol phosphatase (SHIP). Overexpression of SHC increases the level of SHIP phosphorylation on tyrosines in response to ***FLT3*** activation, suggesting that SHC availability is a limiting step for SHIP phosphorylation. This effect is obsd. only if the SHC PTB domain is functional. Interestingly, SHC overexpression in ***FLT3*** -activatable Ba/F3 cells limits ***FLT3*** -dependent cell growth and this effect requires tyrosine 313. Taken together, the present data show that SHC can antagonize cell proliferation induced by ***FLT3*** stimulation and regulate phosphorylation of the SHIP neg. regulator. In addn., our study provides the structural bases for SHC phosphorylation and formation of the SHC/SHIP complex.
RE.CNT 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 52 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1999:644128 CAPLUS
DN 131:332544
TI The use of granulocyte colony-stimulating factor during retroviral ***transduction*** on fibronectin fragment CH-296 enhances gene transfer into ***hematopoietic*** repopulating cells in dogs
AU Goemer, Martin; Bruno, Benedetto; McSweeney, Peter A.; Buron, Greg; Storb, Rainer; Kiem, Hans-Peter
CS Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, 98109-1024, USA
SO Blood (***1999***), 94(7), 2287-2292
CODEN: BLOOAW; ISSN: 0006-4971
PB W. B. Saunders Co.
DT Journal
LA English
AB A competitive repopulation assay in the dog was used to develop improved gene transfer protocols for ***hematopoietic*** stem cell gene therapy. Using this assay, we previously showed improved gene transfer into canine ***hematopoietic*** repopulating cells when CD34-enriched marrow cells were cocultivated on gibbon ape leukemia virus (GALV)-based retrovirus vector-producing cells. In the present study, we have investigated the use of fibronectin fragment CH-296 and 2 growth factor combinations to further improve gene transfer efficiency. CD34-enriched marrow cells from each dog were prestimulated for 24 h and then divided into 3 equal fractions. Two fractions were placed into flasks coated with either CH-296 or bovine serum albumin (BSA) and virus-contg. medium supplemented with growth factors, and protamine sulfate was replaced 4 times over a 48-h period. One fraction was cocultivated on irradiated PG13 (GALV-pseudotype) packaging cells for 48 h. In 2 animals, cells of the different fractions were ***transduced*** in the presence of human FLT-3 ligand (FLT3L), canine stem cell factor (cSCF), and human megakaryocyte growth and development factor (MGDF), and in 2 other dogs, ***transduction*** was performed in the presence of FLT3L, cSCF, and canine granulocyte-colony stimulating factor (cG-CSF). The vectors used contained small sequence differences, allowing differentiation of cells genetically marked by the different vectors. After ***transduction*** , nonadherent and adherent cells from all 3 fractions were pooled and infused into lethally irradiated dogs. Polymerase chain reaction and Southern blot anal. were used to det. the persistence of the transferred vectors in the peripheral blood and marrow cells after transplantation. The highest levels of gene transfer were obtained when cells were ***transduced*** in the presence of FLT3L, cSCF, and cG-CSF (gene transfer levels of more than 10% for more than 8 mo so far). Compared with the 2 animals that received cells ***transduced*** with FLT3L, cSCF, and MGDF, gene transfer levels were significantly higher when dogs received cells that were ***transduced*** in the presence of cG-CSF. ***Transduction*** of CH-296 resulted in gene transfer levels that were at least as high as ***transduction*** by cocultivation. In summary, the overall levels of gene transfer obtained with these conditions should be sufficiently high to allow stem cell gene therapy studies aimed at correcting genetic diseases in dogs as a model for human gene therapy.
RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 53 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1999:585843 CAPLUS
DN 131:309763
TI ***Flt3*** ligand antitumor activity in a murine breast cancer model: a comparison with granulocyte-macrophage colony-stimulating factor and a potential mechanism of action
AU Braun, Stephen E.; Chen, Keyue; Blazar, Bruce R.; Orchard, Paul J.; Sledge, George; Robertson, Michael J.; Broxmeyer, Hal E.; Cornetta, Kenneth
CS Department of Microbiology/Immunology, Indiana University School of Medicine, Indianapolis, IN, 46202, USA
SO Hum. Gene Ther. (***1999***), 10(13), 2141-2151
CODEN: HGTHE3; ISSN: 1043-0342
PB Mary Ann Liebert, Inc.
DT Journal
LA English
AB We have shown that Flk2/ ***Flt3*** ligand (Flt3L)- ***transduced*** tumor vaccine induces transferable T cell protection against a murine breast cancer cell line, but a direct comparison with the potent effector GM-CSF, the activity against pre-established tumors, and the mechanism of antitumor response in this breast cancer model are not known. We compared vaccination with C3L5 cells expressing Flt3L (C3Lt-Flt3L) and GM-CSF (C3L5-GMCSF) by injecting 1.times.104 cells s.c. into the chest wall and then, after 4 wk, challenging the contralateral chest of tumor-free mice with parental C3L5 cells. C3L5-Flt3L and C3L5-GMCSF had reduced in vivo growth rates (25% tumor formation each) compared with 100% tumor formation of C3L5 cells expressing only neomycin phosphotransferase (C3L5-G1N). However, when tumor-free animals were challenged with parental C3L5 cells, C3L5-Flt3L vaccination was significantly better at preventing tumor growth ($p < 0.05$) than C3L5-GMCSF vaccination (33% of C3L5-Flt3L-vaccinated animals developed tumor compared with 77% of C3L5-GMCSF-vaccinated animals). Adoptive transfer of immunity for both vaccines was demonstrated; splenic T cells from tumor-free mice protected naive mice from parental tumor challenge. To simulate minimal disease, parental C3L5 cells at two concns. (high, 5.times.103 cells; or low, 1.times.103 cells) were injected into the contralateral chest wall 4 days prior to treatment with C3L5-G1N or C3L5-Flt3L. C3L5-Flt3L treatment decreased contralateral parental tumor formation (high, 67% tumor free; low, 90% tumor free) compared with C3L5-G1N treatment (high and low, 0% tumor free). Immunodepletion of activated natural killer cells with anti-asialo-GM1 blocked C3L5-Flt3L- and C3L5 plus sol. Flt3L-mediated antitumor activity. Thus, Flt3L- ***transduced*** tumor cells manifest potent antitumor activity, apparently mediated, at least partially, by natural killer cells.
RE.CNT 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 54 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1999:528975 CAPLUS
DN 131:167377
TI Zinc and transition metal-chelating agents for controlling proliferation and differentiation of stem and progenitor cells
IN Peled, Tony; Fibach, Eitan; Treves, Avi; Friedman, Mark M.
PA Gamida Cell Ltd., Israel; Hadasit Medical Research Services and Development Ltd.
SO PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9940783	A1	19990819	WO 1999-US2664	19990208 <--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9926624	A1	19990830	AU 1999-26624	19990208 <--
EP 1069821	A1	20010124	EP 1999-906799	19990208
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, NL, SE, PT				
JP 2002502617	T2	20020129	JP 2000-531059	19990208
WO 2000018885	A1	20000406	WO 1999-IL444	19990817
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9952998	A1	20000417	AU 1999-52998	19990817
EP 1117762	A1	20010725	EP 1999-938494	19990817
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 9914465	A	20011009	BR 1999-14465	19990817
PRAI US 1998-24195	A	19980217		
US 1998-130367	A	19980807		
US 1998-161659	A	19980929		
WO 1999-US2664	W	19990208		
WO 1999-IL444	W	19990817		

AB A method of expanding a population of cells, while at the same time inhibiting differentiation of the cells, is disclosed; the method includes the step of providing the cells with conditions for cell proliferation and, at the same time, for reducing a capacity of the cells in utilizing transition metals. The method can be executed both in vivo and ex vivo .
RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 55 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1999:517816 CAPLUS
DN 131:298142
TI Interleukin-11 (IL-11) enhances clonal proliferation of acute myelogenous leukemia cells with strong expression of the IL-11 receptor .alpha. chain and signal ***transducing*** gp130
AU Kimura, T.; Sakabe, H.; Minamiguchi, H.; Fujiki, H.; Abe, T.; Kaneko, H.; Yokota, S.; Nakagawa, H.; Fujii, H.; Tamaki, H.; Ogawa, H.; Sugiyama, H.;

Sonoda, Y.
CS Department of Hygiene, Kyoto Prefectural University of Medicine, Kyoto, 602, Japan
SO Leukemia (***1999***), 13(7), 1018-1027
CODEN: LEUKED; ISSN: 0887-6924
PB Stockton Press
DT Journal
LA English
AB We examd. the effect of recombinant human interleukin (IL)-11 alone or in combination with various colony-stimulating factors (CSFs), including IL-3, granulocyte/macrophage (GM)-CSF, granulocyte (G)-CSF, stem cell factor (SCF), ***flt3*** ligand (FL), and thrombopoietin (TPO), on colony formation by leukemic progenitor cells (L-CFU) obtained from 33 patients with acute myelogenous leukemia (AML). Leukemic colony formation was found in approx. 70 to 80% of the patients in the presence of at least one of the above CSFs. Although IL-11 alone did not support L-CFU, the growth of these progenitors in the presence of other cytokines was enhanced by IL-11 in 16 out of 33 patients and it showed a synergistic action with G-CSF in 12 of them. This synergistic action occurred in seven out of nine M5 patients (French-American-British (FAB) classification). A single cell clone-sorting expt. clearly demonstrated that this synergistic effect was operative at the single progenitor cell level. The no. of leukemic cells proliferating in the presence of G-CSF+IL-11 was significantly higher than in the presence of G-CSF alone, suggesting that IL-11 recruited dormant leukemic progenitors into the cell cycle. Flow cytometric anal. revealed that all types of AML blast cells (M0,apprx.M6) ubiquitously expressed gp130, although the level of expression was significantly higher in M5 cells. In contrast, expression of the IL-11 receptor .alpha. chain (IL-11R.alpha.) varied between FAB types. Blast cells obtained from M1, M3 and M5 patients showed higher levels of expression, with M5 cells showing the strongest expression. Interestingly, the leukemic progenitor cells for which proliferation was synergistically enhanced by IL-11 had significantly higher expression of both IL-11R.alpha. and gp130. These results suggest that administration of IL-11 in vivo may stimulate the proliferation of leukemic progenitor cells, particularly M5 cells, in the presence of G-CSF, and that the responsiveness of L-CFU to IL-11 may be predicted by a simple receptor assay.
RE.CNT 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 75-80

L12 ANSWER 75 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1997:269199 CAPLUS
DN 126:312642
TI Coexpression of flt-3 ligand/flt-3 and SCF/c-kit signal ***transduction*** systems in bile-duct-ligated Sl and W mice
AU Omori, Masako; Omori, Nobuhiko; Evarts, Ritva P.; Teramoto, Tadahisa; Thorgeirsson, Snorri S.
CS Laboratory of Experimental Carcinogenesis, Division of Basic Sciences, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892-4255, USA
SO Am. J. Pathol. (***1997***), 150(4), 1179-1187
CODEN: AJPAA4; ISSN: 0002-9440
PB American Society for Investigative Pathology
DT Journal
LA English
AB Stem cell factor (SCF) and its receptor c-kit constitute an important signal ***transduction*** system regulating cell growth and differentiation in ***hematopoiesis*** , gametogenesis, and melanogenesis. Recently, it was have demonstrated that both SCF and c-kit are expressed in the bile duct epithelial cells of the rat liver and are highly up-regulated during activation of the normally dormant hepatic stem cell compartment. In the present study, the authors used sl/sld and wt.Awt.v mice, which have mutation of either SCF or c-kit, to study the possible involvement of the SCF/c-kit system in the bile duct proliferation. Bile duct ligation was performed to induce the proliferation of bile duct epithelial cells. The transcripts for both SCF and c-kit were clearly increased after bile duct ligation in both control and mutant mice. Moreover, both Sl and W mice responded to the bile duct ligation, similar to the control mice, by developing new bile ducts. Recently, a novel tyrosine kinase receptor, flt-3 receptor, has been identified in the fetal liver. It has been reported that the flt-3 ligand (FL)/flt-3 system can synergize with the SCF/c-kit system and stimulate the proliferation of ***hematopoietic*** cells. Therefore, the authors hypothesized that the FL/flt-3 system might compensate for the compromised SCF/c-kit system in the liver of Sl and W mice. The expression of both FL and flt-3 were significantly increased in bile-duct-ligated liver from both normal and mutant mice, and the transcripts for the flt-3 receptor were selectively located on bile duct epithelial cells. Based on these results, the authors postulate the existence of a compensatory/additive function between the FL/flt-3 and the SCF/c-kit signal ***transduction*** systems in hepatic cell biol.

L12 ANSWER 76 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1997:119189 CAPLUS
DN 126:130574
TI Serum-free media compositions for expansion of ***hematopoietic*** progenitor and/or stem cells
IN Tsao, Mary C.; Tanaka, Wallace W.
PA Sandoz Ltd., Switz.; Systemix, Inc.; Sandoz-Erfindungen Verwaltungsgesellschaft Mbh; Sandoz-Patent-Gmbh
SO PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9640868	A1	19961219	WO 1996-EP2454	19960606 <--
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN				
AU 9661253	A1	19961230	AU 1996-61253	19960606 <--
PRAI US 1995-484514		19950607		
WO 1996-EP2454		19960606		

AB The invention provides compns. suitable for serum-free liq. culture, expansion, ***transduction*** , cryopreservation, etc. of human ***hematopoietic*** progenitor and stem cells. CD34+Thy-1+LIN- cells were isolated from adult bone marrow or mobilized peripheral blood and expanded in culture media comprising various components disclosed in this invention.

L12 ANSWER 77 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1996:756736 CAPLUS
DN 126:29834
TI Retroviral ***transduction*** of human progenitor cells: use of granulocyte colony-stimulating factor plus stem cell factor to mobilize progenitor cells in vivo and stimulation by ***Flt3*** /Flk-2 ligand in vitro
AU Elwood, Ngaire J.; Zogos, Helen; Willson, Tracy; Begley, C. Glenn
CS Rotary Bone Marrow Res. Lab., Royal Melbourne Hospital, Parkville, Australia
SO Blood (***1996***), 88(12), 4452-4462
CODEN: BLOOAW; ISSN: 0006-4971
PB Saunders
DT Journal
LA English
AB The clin. application of gene transfer is hindered by the availability of the multipotential stem cells and the difficulty in obtaining efficient retroviral ***transduction*** . To assess potential means by which gene transfer into human hemopoietic stem cells might be enhanced, the retroviral ***transduction*** efficiency of human bone marrow cells (BM) or peripheral blood progenitor cells (PBPC) was compared at multiple time points after in vivo administration of granulocyte colony-stimulating factor (G-CSF). This was further compared with the ***transduction*** efficiency of cells mobilized with G-CSF plus stem cell factor (SCF) in a cohort of patients randomized to receive either one or two growth factors and with normal BM function. Using the LNL6 retrovirus, retroviral ***transduction*** efficiencies of up to 19% were obsd. for both PBPC and BM (n = 26 patients). There was at least a 100-fold increase in PBPC with G-CSF alone and a further 30-fold increase in the total no. of progenitor cells available for retroviral ***transduction*** using the combination of SCF plus G-CSF. However, pretreatment of patients with G-CSF with or without SCF did not enhance the retroviral infectability of growth factor-mobilized progenitor cells. The effect of the growth factor, Flk-2/ ***Flt3*** ligand (FL), was also examd. with respect to retroviral ***transduction*** efficiency of human progenitor cells. FL plus IL-3 in vitro increased the retroviral ***transduction*** efficiency up to eightfold compared with results obsd. using other combinations of cytokines tested (P < .001). These findings have clin. implications both for increasing the no. of target cells for in vivo gene-marking/gene-therapy studies and improving the efficiency of gene transfer.

L12 ANSWER 78 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1995:444160 CAPLUS
DN 122:207009
TI ***Flt3*** receptor ligand (***flt3*** -L), cloning and expression of cDNA for ***flt3*** -L, and use of ***flt3*** -L to influence ***hematopoietic*** or stem cells
IN Lyman, Stewart D.; Beckmann, M. Patricia
PA Immunex Corp., USA
SO Eur. Pat. Appl., 33 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 627487	A2	19941207	EP 1994-303575	19940519 <--
EP 627487	A3	19960821		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
US 5554512	A	19960910	US 1994-243545	19940511 <--
AU 9469877	A1	19941220	AU 1994-69877	19940512 <--
AU 683472	B2	19971113		
CN 1125479	A	19960626	CN 1994-192225	19940512 <--
BR 9407073	A	19960827	BR 1994-7073	19940512 <--
JP 08511251	T2	19961126	JP 1994-500715	19940512 <--
ZA 9403490	A	19950123	ZA 1994-3490	19940520 <--
FI 9505646	A	19960123	FI 1995-5646	19951123 <--
NO 9504735	A	19960123	NO 1995-4735	19951123 <--
PRAI US 1993-68394	A	19930524		
US 1993-106463	A	19930812		
US 1993-111758	A	19930825		
US 1993-162407	A	19931203		
US 1994-209502	A	19940307		
US 1994-243545	A	19940511		
WO 1994-US5365	W	19940512		

AB Ligands for ***flt3*** receptors capable of ***transducing*** self-renewal signals to regulate the growth, proliferation or differentiation of progenitor cells and stem cells are disclosed. The invention is directed to ***flt3*** -L as an isolated protein, the DNA encoding the ***flt3*** -L, host cells transfected with the cDNA's encoding ***flt3*** -L, compns. comprising ***flt3*** -L, methods of improving gene transfer to a mammal using ***flt3*** -L, and methods of improving transplantations using flt-L. ***Flt3*** -L find use in treating patients with anemia, AIDS and various cancers. The cDNA's for both murine and human ***flt3*** -L were cloned. A sol. ***flt3*** -L was prepd. with yeast and monoclonal antibodies to the ligand were produced. ***Flt3*** -L stimulated ***hematopoiesis*** ; stimulation with ***flt3*** -L and interleukin-7 together was 4-fold greater. ***Flt3*** -L was shown to stimulate proliferation of erythroid cells in spleen and to stimulate proliferation of T cells and early B cells.

L12 ANSWER 79 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1995:12468 CAPLUS
DN 122:52454
TI Fms-like tyrosine kinase 3 catalytic domain can ***transduce*** a proliferative signal in FDC-P1 cells that is quantitatively similar to the signal delivered by c-Fms
AU Rossner, Michael T.; McArthur, Grant A.; Allen, John D.; Metcalf, Donald
CS Walter and Eliza Hall Inst. Med. Res., Parkville, 3050, Australia
SO Cell Growth Differ. (***1994***), 5(5), 549-55
CODEN: CGDIE7; ISSN: 1044-9523
DT Journal
LA English
AB A full length clone of murine fms-like tyrosine kinase 3 [***flt3*** ,

also known as fetal liver kinase 2 (flk2)] was constructed from sequences obtained from a brain complementary DNA (cDNA) library and from cDNA prepd. from the cell line Tikaut. In the absence of a ligand to study the function of ***Flt3*** , a chimeric mol. was constructed comprising the extracellular domain of murine c-Fms and the transmembrane and cytoplasmic domains of ***Flt3*** . A plasmid encoding the chimeric receptor was cotransfected along with a plasmid conferring neomycin resistance into FDC-P1 cells that do not normally express c-fms or ***flt3*** and require granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin 3 for growth. Two types of clones were obtained following selection in GM-CSF and G418. Two of seven clones had the capacity for M-CSF-dependent colony formation in semisolid medium, indicating that the cytoplasmic domain of ***Flt3*** can ***transduce*** a proliferative signal. From the remaining clones, M-CSF-dependent clonogenic cells could be selected by prior bulk liq. culture in M-CSF. It has been shown previously that the GM-CSF-dependent proliferative capacity is strongly inhibited by M-CSF in FDC-P1 cells engineered to express full length c-fms. This phenomenon was also obsd. with FD/fms-***flt3*** cells that were clonogenic in M-CSF. Stimulation of FD/fms or FD/fms- ***flt3*** cells in liq. culture by M-CSF caused differentiation of a small proportion of cells along the myelomonocytic pathway which was enhanced by the combination of M-CSF and GM-CSF. The similarity of the response of cells bearing either c-Fms or the Fms/***Flt3*** chimeric receptor to stimulation by M-CSF suggests that ***Flt3*** and c-Fms function through similar signaling pathways.

L12 ANSWER 80 OF 80 CAPLUS COPYRIGHT 2002 ACS
AN 1994:554127 CAPLUS
DN 121:154127
TI Substrate specificities and identification of a putative binding site for PI3K in the carboxy tail of the murine ***Flt3*** receptor tyrosine kinase
AU Rottapel, Robert; Turck, Christoph W.; Casteran, Nathalie; Liu, Xinguan; Birnbaum, Daniel; Pawson, Tony; Dubreuil, Patrice
CS Mol. Hematol. Lab., INSERM, Marseille, 13009, Fr.
SO Oncogene (***1994***), 9(6), 1755-65
CODEN: ONCNES; ISSN: 0950-9232
DT Journal
LA English
AB ***Flt3*** is a receptor protein tyrosine kinase (RTK) structurally related to the CSF-1R receptor (encoded by the c-fms locus), Kit receptor, and the platelet-derived growth factor receptor kinases and is restricted in its expression to ***hematopoietic*** precursor populations and several distinct cell types within the central nervous system. Although the ligand for ***Flt3*** has recently been identified, the developmental function of ***Flt3*** within these tissues has not yet been described. In order to examine the signalling properties of this receptor, the authors previously constructed a chimeric mol. contg. the extracellular domain of CSF-1R fused to the transmembrane and cytoplasmic domain of mouse ***Flt3*** (FF3). The ability of the FF3 to directly assoc. with or tyrosine-phosphorylate specific cytoplasmic signaling mols. in vivo was examd. Proteins GAP, Vav, Shc, and to a lesser extent phosphatidylinositol phospholipase C-.gamma. became tyrosine-phosphorylated but no in vivo assocn. with the receptor was detectable. FF3 assocd. with phosphatidylinositol 3-kinase (PI3K) activity and the SH2 domains of proteins p85 and Grb-2. Phosphopeptide competition expts. suggested that the PI3K binding site is located outside of the kinase insert in the C-terminal tail of the receptor.

=> d his

(FILE 'HOME' ENTERED AT 12:10:26 ON 31 JAN 2002)

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 12:10:51 ON 31 JAN 2002
L1 244 S RD114
L2 61 S L1 AND VECTOR?
L3 32 DUP REM L2 (29 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 12:14:39 ON 31 JAN 2002

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 12:47:19 ON 31 JAN 2002
L4 23 S RETRONECTIN
L5 18 DUP REM L4 (5 DUPLICATES REMOVED)
L6 0 S TRASDUCT? AND STEM CELL? AND FLT!3
L7 0 S TRANSDUCT? AND STEM CELL? AND FLT!3
L8 0 S TRANSDUC? AND STEM CELL? AND FLT!3
L9 0 S TRANSDUC? AND HEMATOPOIE? AND FLT!3
L10 220 S TRANSDUC? AND HEMATOPOIE? AND FLT3
L11 145 DUP REM L10 (75 DUPLICATES REMOVED)
L12 80 S L11 AND PY<2000

=> s l3 and (oncovir? or lentivir?)
L13 4 L3 AND (ONCOVIR? OR LENTIVIR?)

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14 4 DUP REM L13 (0 DUPLICATES REMOVED)

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L14 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2002 ACS
AN 2001:676635 CAPLUS
DN 135:236393
TI Highly efficient gene transfer into human repopulating stem cells by ***RD114*** envelope protein pseudotyped retroviral ***vector*** particles which pre-adsorb on retronectin-coated plates
IN Kelly, Patrick F.; Vanin, Elio F.
PA St. Jude Children's Research Hospital, USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001066150	A2	20010913	WO 2001-US7212	20010307

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
US 2001051375 A1 20011213 US 2001-801302 20010307
PRAI US 2000-187534 P 20000307
AB The present invention relates to a method for efficiently introducing exogenous genes into stem cells, particularly human stem cells. The method optionally includes the steps of inducing the proliferation of target cells by pre-stimulation with cytokines and/or growth factors, followed by incubating these cells with ***RD114*** -pseudotyped ***vector*** particles. In a specific embodiment, the ***vector*** particles are retronectin-immobilized or ultracentrifugation-concd. retroviral ***vector*** particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein. The present invention further discloses a method for somatic gene therapy, which can be used for various therapeutic applications and involves introducing a gene of interest contained within the retroviral genome into human repopulating stem cells followed by introducing these cells into a human host. Finally, the present invention discloses a method for monitoring the efficiency of the stem cell-mediated gene transfer based on detecting the presence of the genes (or the expression products) of the retroviral ***vector*** in various stem cell-derived lineages of the host.

L14 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2002 ACS
AN 2000:210402 CAPLUS
DN 132:247121
TI Pseudotyped retroviral ***vector*** gene transfer system for hemophilia in vivo gene therapy
IN Vandendriessche, Thierry; Chuah, Marinee K. L.
PA Vlaams Interuniversitair Instituut Voor Biotechnologie Vzw, Belg.
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000017375	A2	20000330	WO 1999-EP7384	19990921
WO 2000017375	A3	20000727		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
AU 9964681 A1 20000410 AU 1999-64681 19990921
PRAI EP 1998-203203 A 19980923
WO 1999-EP7384 W 19990921
AB The present invention relates to a gene transfer system, preferably pseudotyped retroviral ***vectors*** allowing stable expression of biol. active proteins at therapeutic, physiol. or supraphysiol. levels. The invention relates particularly to a method to treat hemophilia A or B using said ***vectors*** to express coagulation factors by in vivo gene therapy. Pseudotyping the retroviral ***vectors*** prevents induction of inhibitory or neutralizing antibody against the biol. active protein expressed in the animal model or the patient injected with the ***vector*** . VSV-G pseudotyped MFG-FVIIIDB retroviral ***vector*** was generated and injected i.v. into factor VIII (FVIII)-deficient mice. Long term, high level expression of human FVIII was detected in 6 of 13 mice, without the detection of human FVIII-specific inhibitory antibodies. These mice expressing a high level of human FVIII survived an otherwise lethal tail-clipping, demonstrating phenotypic correction of hemophilia A in FVIII-deficient mice.

L14 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:311867 BIOSIS
DN PREV200100311867
TI Improved transduction of human primitive hematopoietic cells with a ***lentiviral*** ***vector*** pseudotyped with the envelope protein of endogenous feline leukemia virus (***RD114*** .
AU Hanawa, Hideki (1); Kelly, Patrick F. (1); Nathwani, Amit C. (1); Nienhuis, Arthur W. (1); Vanin, Elio F. (1)
CS (1) Division of Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 524a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB ***Lentiviral*** ***vectors*** based on HIV have inherent advantages in transducing non-dividing cells in that their pre-integration nucleoprotein complex is relatively stable and able to transverse the nuclear membrane without mitosis. Most HIV based ***vector*** systems studied to date have utilized the envelope protein of the vesicular stomatitis virus (VSV-G). We have found that the envelope protein of endogenous feline leukemia virus (***RD114***), when used to pseudotype murine oncoretroviral ***vectors*** , yields particles that very efficiently transduce primitive hematopoietic cells from cord blood, including those which establish human hematopoiesis in immunodeficient mice (Kelly et al, Blood 96:1206, 2000). ***Lentiviral*** ***vector*** particles pseudotyped with ***RD114*** envelope were produced by co-transfecting 293T cells with a ***vector*** plasmid which encodes the green fluorescent protein (GFP), a plasmid encoding the HIV matrix and enzyme proteins, a plasmid encoding the HIV tat and rev proteins, and either a plasmid encoding the VSV-G or ***RD114*** envelope protein. ***Vector*** production as assessed by p24 measurement in conditioned medium was essentially equivalent (VSV-G = 930ng/ml and ***RD114*** = 1240ng/ml). The titer of VSV-G particles was 30-fold higher on HeLa cells. At a multiplicity of infection (MOI) of 15 (HeLa titers) without prestimulation, transduction of cord blood CD34+ cells averaged 51.5% (range 15-78%) with ***RD114*** pseudotyped HIV ***vector*** particles whereas the corresponding values were 5.8% (range 2-9%) with the HIV ***vector*** pseudotyped with VSV-G or less than 1% with murine oncoretroviral ***vector*** particles pseudotyped with

RD114 . With 48 hours of prestimulation, ***RD114*** pseudotyped ***lentiviral*** particles were more efficient than VSV-G pseudotyped particles at transducing cord blood (87% vs. 38%) or peripheral blood (51% vs. 21%) CD34+ cells. Using a second design, cells were exposed to equivalent numbers of ***vector*** particles based on p24 measurement. With this design, 72% of cord blood, CD34+ cells and 34% of CD34+, CD38- cells were transduced with ***RD114*** pseudotyped ***vector*** particles compared to 19% and 8%, respectively, with VSV-G pseudotyped ***lentiviral*** ***vector*** particles. Our results indicate that the ***RD114*** envelope will effectively pseudotype HIV based ***lentiviral*** ***vectors*** and suggest that ***RD114*** pseudotyped ***lentiviral*** ***vector*** particles transduce primitive human hematopoietic cells at greater efficiency than do VSV-G pseudotyped ***lentiviral*** ***vector*** particles.

L14 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:165327 BIOSIS
DN BA93:87652
TI RETROVIRAL PSEUDOTYPES PRODUCED BY RESCUE OF A MOLONEY MURINE LEUKEMIA VIRUS ***VECTOR*** BY C-TYPE BUT NOT D-TYPE RETROVIRUSES.
AU TAKEUCHI Y; SIMPSON G; VILE R G; WEISS R A; COLLINS M K L
CS CHESTER BEATTY LABS., INST. CANCER RES., 237 FULHAM ROAD, LONDON SW3 6JB, UK.
SO VIROLOGY, (1992) 186 (2), 792-794.
CODEN: VIRLAX. ISSN: 0042-6822.
FS BA; OLD
LA English
AB Human HOS cells containing a Moloney murine leukemia virus (Mo-MLV) recombinant genome were infected by a panel of retroviruses. The C-type viruses simian sarcoma associated virus, feline leukemia virus subgroup B, and the feline endogenous virus ***RD114*** were able to form pseudotypes with the Mo-MLV genome, which transferred a selectable marker gene to target cells; however, Human T cell leukemia virus-1 and the D-type viruses Mason-Pfizer monkey virus and simian retrovirus-1 failed to rescue the Mo-MLV ***vector*** . Further characterization of the ***RD114*** pseudotype demonstrated that it retained the receptor specificity of ***RD114*** and will therefore prove useful in receptor characterization.

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NEWS 3 Oct 09 Korean abstracts now included in Derwent World Patents Index
NEWS 4 Oct 09 Number of Derwent World Patents Index updates increased
NEWS 5 Oct 15 Calculated properties now in the REGISTRY/ZREGISTRY File
NEWS 6 Oct 22 Over 1 million reactions added to CASREACT
NEWS 7 Oct 22 DGENE GETSIM has been improved
NEWS 8 Oct 29 AASD no longer available
NEWS 9 Nov 19 New Search Capabilities USPATFULL and USPAT2
NEWS 10 Nov 19 TOXCENTER(SM) - new toxicology file now available on STN
NEWS 11 Nov 29 COPPERLIT now available on STN
NEWS 12 Nov 29 DWPI revisions to NTIS and US Provisional Numbers
NEWS 13 Nov 30 Files VETU and VETB to have open access
NEWS 14 Dec 10 WPINDEX/WPIDS/WPIX New and Revised Manual Codes for 2002
NEWS 15 Dec 10 DGENE BLAST Homology Search
NEWS 16 Dec 17 WELDASEARCH now available on STN
NEWS 17 Dec 17 STANDARDS now available on STN
NEWS 18 Dec 17 New fields for DPCI
NEWS 19 Dec 19 CAS Roles modified
NEWS 20 Dec 19 1907-1946 data and page images added to CA and CAplus
NEWS 21 Jan 25 BLAST(R) searching in REGISTRY available in STN on the Web
NEWS 22 Jan 25 Searching with the P indicator for Preparations
NEWS 23 Jan 29 FSTA has been reloaded and moves to weekly updates
NEWS 24 Feb 01 DKILIT now produced by FIZ Karlsruhe and has a new update frequency

NEWS EXPRESS February 1 CURRENT WINDOWS VERSION IS V6.0d, CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP), AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
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FILE 'HOME' ENTERED AT 11:08:13 ON 05 FEB 2002

=> FIL BIOSIS CAPLUS EMBASE
COST IN U.S. DOLLARS SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST 0.15 0.15

FILE 'BIOSIS' ENTERED AT 11:08:30 ON 05 FEB 2002
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=> s RD114
L1 244 RD114

=> s l1 or FLYRD18
L2 261 L1 OR FLYRD18

=> s l2 and stem cell? and lentivir?
L3 1 L2 AND STEM CELL? AND LENTIVIR?

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 1 ANSWERS - CONTINUE? Y/(N):y

L3 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
AN 2001:676635 CAPLUS
DN 135:236393
TI Highly efficient gene transfer into human repopulating ***stem*** ***cells*** by ***RD114*** envelope protein pseudotyped retroviral vector particles which pre-adsorb on retronectin-coated plates
IN Kelly, Patrick F.; Vanin, Elio F.
PA St. Jude Children's Research Hospital, USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001066150	A2	20010913	WO 2001-US7212	20010307

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
US 2001051375 A1 20011213 US 2001-801302 20010307
PRAI US 2000-187534 P 20000307

AB The present invention relates to a method for efficiently introducing exogenous genes into ***stem*** ***cells*** , particularly human ***stem*** ***cells*** . The method optionally includes the steps of inducing the proliferation of target cells by pre-stimulation with cytokines and/or growth factors, followed by incubating these cells with ***RD114*** -pseudotyped vector particles. In a specific embodiment, the vector particles are retronectin-immobilized or ultracentrifugation-concd. retroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein. The present invention further discloses a method for somatic gene therapy, which can be used for various therapeutic applications and involves introducing a gene of interest contained within the retroviral genome into human repopulating ***stem*** ***cells*** followed by introducing these cells into a human host. Finally, the present invention discloses a method for monitoring the efficiency of the ***stem*** ***cell*** -mediated gene transfer based on detecting the presence of the genes (or the expression products) of the retroviral vector in various ***stem*** ***cell*** -derived lineages of the host.

=> d his

(FILE 'HOME' ENTERED AT 11:08:13 ON 05 FEB 2002)

FILE 'BIOSIS, CAPLUS, EMBASE' ENTERED AT 11:08:30 ON 05 FEB 2002
L1 244 S RD114
L2 261 S L1 OR FLYRD18
L3 1 S L2 AND STEM CELL? AND LENTIVIR?

=> s l2 and stem cell?
L4 15 L2 AND STEM CELL?

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 9 DUP REM L4 (6 DUPLICATES REMOVED)

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y

L5 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2002 ACS
AN 2001:676635 CAPLUS
DN 135:236393
TI Highly efficient gene transfer into human repopulating ***stem*** ***cells*** by ***RD114*** envelope protein pseudotyped retroviral vector particles which pre-adsorb on retronectin-coated plates
IN Kelly, Patrick F.; Vanin, Elio F.
PA St. Jude Children's Research Hospital, USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001068150	A2	20010913	WO 2001-US7212	20010307
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2001051375	A1	20011213	US 2001-801302	20010307
PRAI US 2000-187534	P	20000307		

AB The present invention relates to a method for efficiently introducing exogenous genes into ***stem*** ***cells*** , particularly human ***stem*** ***cells*** . The method optionally includes the steps of inducing the proliferation of target cells by pre-stimulation with cytokines and/or growth factors, followed by incubating these cells with ***RD114*** -pseudotyped vector particles. In a specific embodiment, the vector particles are retronectin-immobilized or ultracentrifugation-concd. retroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein. The present invention further discloses a method for somatic gene therapy, which can be used for various therapeutic applications and involves introducing a gene of interest contained within the retroviral genome into human repopulating ***stem*** ***cells*** followed by introducing these cells into a human host. Finally, the present invention discloses a method for monitoring the efficiency of the ***stem*** ***cell*** -mediated gene transfer based on detecting the presence of the genes (or the expression products) of the retroviral vector in various ***stem*** ***cell*** -derived lineages of the host.

L5 ANSWER 2 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:415218 BIOSIS
DN PREV200100415218
TI ***RD114*** -Pseudotyped oncoretroviral vectors: Biological and physical properties.
AU Kelly, Patrick F.; Carrington, Jody; Nathwani, Amit; Vanin, Elio F. (1)
CS (1) Division of Experimental Hematology, Department of Hematology/Oncology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN, 38105: elio.vanin@stjude.org USA
SO Orlic, Donald; Bruemmendorf, Tim H.; Sharkis, Saul J.; Kanz, Lothar. Annals of the New York Academy of Sciences, (June, 2001) Vol. 938, pp. 262-277. Annals of the New York Academy of Sciences. Hematopoietic stem cells 2000: Basic and clinical sciences: Third International Conference. print.
Publisher: New York Academy of Sciences 2 East 63rd Street, New York, NY, 10021, USA.
Meeting Info.: Conference on Hematopoietic Stem Cells: Genetics and Medicine Tubingen, Germany September 14-18, 2000
ISSN: 0077-8923. ISBN: 1-57331-295-9 (cloth), 1-57331-296-7 (paper).
DT Book; Conference
LA English
SL English

L5 ANSWER 3 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2001:526085 BIOSIS
DN PREV200100526085
TI Engraftment of NOD/SCID mice with human CD34+ cells transduced by concentrated oncoretroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein.
AU Gatlin, Joel; Melkus, Michael W.; Padgett, Angela; Kelly, Patrick F.; Garcia, J. Victor (1)
CS (1) Division of Infectious Diseases Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas, Y9.206, Dallas, TX, 75390-9113: victor.garcia@utsouthwestern.edu USA
SO Journal of Virology, (October, 2001) Vol. 75, No. 20, pp. 9995-9999. print.
ISSN: 0022-538X.
DT Article
LA English
SL English
AB Oncoretrovirus vectors pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein produced by the ***FLYRD18*** packaging cell line have previously been shown to transduce human hematopoietic progenitor cells with a greater efficiency than similar amphotropic envelope-pseudotyped vectors. In this report, we describe the production and efficient concentration of ***RD114*** -pseudotyped murine leukemia virus (MLV)-based vectors. Following a single round of centrifugation, vector supernatants were concentrated approximately 200-fold with a 50 to 70% yield. Concentrated vector stocks transduced prestimulated human CD34+ (hCD34+) cells with approximately 69% efficiency (n = 7, standard deviation = 4.4%) using a single addition of vector at a low multiplicity of infection (MOI = 5). Introduction of transduced hCD34+ cells into irradiated NOD/SCID recipients resulted in multilineage engraftment with long-term transgene expression. These data demonstrate that ***RD114*** -pseudotyped MLV-based vectors can be efficiently concentrated to high titers and that hCD34+ cells transduced with concentrated vector stocks retain in vivo repopulating potential. These results highlight the potential of ***RD114*** -pseudotyped oncoretrovirus vectors for future clinical implementation in hematopoietic ***stem*** ***cell*** gene transfer.

L5 ANSWER 4 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:512683 BIOSIS
DN PREV200100512683
TI Sustained multilineage gene persistence and expression in dogs transplanted with CD34+ marrow cells transduced by ***RD114*** -pseudotype oncoretrovirus vectors.
AU Goerner, Martin; Horn, Peter A.; Peterson, Laura; Kurre, Peter; Storb, Rainer; Rasko, John E. J.; Kiem, Hans-Peter (1)
CS (1) Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D1-100, Seattle, WA, 98109-1024: hkiem@fhcrc.org USA
SO Blood, (October 1, 2001) Vol. 98, No. 7, pp. 2065-2070. print.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB Previous studies have shown that the choice of envelope protein (pseudotype) can have a significant effect on the efficiency of retroviral gene transfer into hematopoietic ***stem*** ***cells*** . This study used a competitive repopulation assay in the dog model to evaluate oncoretroviral vectors carrying the envelope protein of the endogenous feline virus, ***RD114*** . CD34-enriched marrow cells were divided into equal aliquots and transduced with vectors produced by the ***RD114*** -pseudotype packaging cells FLYRD (LgGLSN and LNX) or by the gibbon ape leukemia virus (GALV)-pseudotype packaging cells PG13 (LNY). A total of 5 dogs were studied. One dog died because of infection before sustained engraftment could be achieved, and monitoring was discontinued after 9 months in another animal that had very low overall gene-marking levels. The 3 remaining animals are alive with follow-ups at 11, 22, and 23 months. Analyses of gene marking frequencies in peripheral blood and marrow by polymerase chain reaction revealed no significant differences between the ***RD114*** and GALV-pseudotype vectors. The LgGLSN vector also contained the enhanced green fluorescent protein (GFP), enabling us to monitor proviral expression by flow cytometry. Up to 10% of peripheral blood cells expressed GFP shortly after transplantation and approximately 6% after the longest follow-up of 23 months. Flow cytometric analysis of hematopoietic sub-populations showed that most of the GFP-expressing cells were granulocytes, although GFP-positive lymphocytes and monocytes were also detected. In summary, these results show that ***RD114*** -pseudotype oncoretroviral vectors are able to transduce hematopoietic long-term repopulating cells and, thus, may be useful for human ***stem*** ***cell*** gene therapy.

L5 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
AN 2001:549788 CAPLUS
TI ***RD114*** -pseudotyped oncoretroviral vectors: Biological and physical properties
AU Kelly, Patrick F.; Carrington, Jody; Nathwani, Amit; Vanin, Elio F.
CS Division of Experimental Hematology, Department of Hematology/Oncology, St. Jude Children's Research Hospital, Memphis, TN, 38101, USA
SO Ann. N. Y. Acad. Sci. (2001), 938(Hematopoietic Stem Cells 2000), 262-277
CODEN: ANYAA9; ISSN: 0077-8923
PB New York Academy of Sciences
DT Journal
LA English
AB Limited functional expression of the viral envelope receptor is a recognized barrier to efficient oncoretroviral mediated gene transfer. To circumvent this barrier we evaluated a no. of envelope proteins with respect to gene transfer efficiency into primitive human hematopoietic ***stem*** ***cell*** populations. We obsd. that oncoretroviral vectors pseudotyped with the envelope protein of feline endogenous virus (***RD114***) could efficiently transduce human repopulating cells capable of establishing multilineage hematopoiesis in immunodeficient mice after a single exposure to ***RD114*** -pseudotyped vector. Comparable rates of gene transfer with amphotropic and GALV-pseudotyped vectors have been reported, but only after multiple exposures to the viral supernatant. Oncoretroviral vectors pseudotyped with the ***RD114*** or the amphotropic envelopes had similar stability in vitro, indicating that the increased efficiency in gene transfer is at the receptor level likely due to increased receptor expression or an increased receptor affinity for the ***RD114*** envelope. We also found that ***RD114*** -pseudotype vectors can be efficiently concd., thereby removing any adverse effects of the conditioned media to the long-term repopulating potential of the target human hematopoietic ***stem*** ***cell*** . These studies demonstrate the potential of ***RD114*** -pseudotyped vectors for clin. use.
RE.CNT 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2000:415630 BIOSIS
DN PREV200000415630
TI Highly efficient gene transfer into cord blood nonobese diabetic/severe combined immunodeficiency repopulating cells by oncoretroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein.
AU Kelly, Patrick F. (1); Vandergriff, Jody; Nathwani, Amit; Nienhuis, Arthur W.; Vanin, Elio F.
CS (1) Division of Experimental Hematology, St Jude Children's Research Hospital, 332 N Lauderdale, Room D-4026, Memphis, TN, 38105 USA
SO Blood, (August 15, 2000) Vol. 96, No. 4, pp. 1208-1214. print.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB Limited expression of the amphotropic envelope receptor is a recognized barrier to efficient oncoretroviral vector-mediated gene transfer. Human hematopoietic cell lines and cord blood-derived CD34+ and CD34+, CD38- cell populations and the progenitors contained therein were transduced far more efficiently with oncoretroviral particles pseudotyped with the envelope protein of feline endogenous virus (***RD114***) than with conventional amphotropic vector particles. Similarly, human repopulating cells from umbilical cord blood capable of establishing hematopoiesis in

immunodeficient mice were efficiently transduced with ***RD114*** -pseudotyped particles, whereas amphotropic particles were ineffective at introducing the proviral genome. After only a single exposure of CD34+ cord blood cells to ***RD114*** -pseudotyped particles, all engrafted nonobese diabetic/severe combined immunodeficiency mice (15 of 15) contained genetically modified human bone marrow cells. Human cells that were positive for enhanced green fluorescent protein represented as much as 90% of the graft. The use of ***RD114*** -pseudotyped vectors may be advantageous for therapeutic gene transfer into hematopoietic ***stem*** ***cells*** .

L5 ANSWER 7 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302193 BIOSIS
DN PREV200100302193
TI Multilineage transduction of non-human primate CD34+ hematopoietic cells using RD-114 pseudotyped oncoretroviruses.
AU Kelly, Patrick F. (1); Bonifacino, Aylin C.; Carrington, Jody A. (1); Agricola, Brian A.; Metzger, Mark E.; Kluge, Kim A.; Nienhuis, Arthur W. (1); Donahue, Robert E.; Vanin, Elio F. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 525a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The relative quiescence of the hematopoietic ***stem*** ***cell*** (HSC) and the low level of viral receptor expression are known to contribute to the low efficiency of retroviral gene transfer into HSCs of large animals and humans. We have previously reported that ***RD114*** -pseudotyped retroviruses could efficiently transduce cord blood CD34+ cells after 24-48 hours pre-stimulation and a single exposure to the viral particles preloaded onto RetroNectin-coated plates. Based on these results we evaluated gene transfer of ***RD114*** -pseudotyped murine retroviruses using non-human primate CD34+ peripheral blood (PB) cells in the rhesus autologous transplant model. SCF/G-CSF-mobilized rhesus monkey PB were collected and enriched for CD34+ cells. These cells were cultured in serum-containing medium with high concentrations of SCF, FLT-3 and IL-6 and exposed to ***RD114*** -pseudotyped particles preloaded onto RetroNectin-coated plates at 48 hours and 72 hours. After 96 hours in culture, cells were harvested and infused into irradiated recipients (2 X 500 cGy, n=5). The transduction efficiency of the infused cells was 35-55% based on EGFP expression. In all animals we have observed multilineage engraftment with persistence of EGFP expression after 6-8 weeks post-transplantation, a result that was not achieved with a similar construct pseudotyped with the amphotropic envelope protein. In the first animal transplanted, we observed high levels of multilineage engraftment of EGFP+ cells (as high as 98% in granulocytes) over the first 20 weeks post transplantation. After 26 weeks multilineage expression has stabilized at 8-10%. Serial genomic southern analysis for both proviral integrity and integration site indicated that vector silencing was not occurring and that the engraftment of gene modified cells was oligoclonal. The second recipient displayed similar kinetics but died from transplant related complications 8 weeks post-transplantation. Subsequent animals have achieved lower levels of EGFP expression (1-3%) suggesting that transduction conditions using this pseudotype remains to be optimized. These results suggest oncoretroviral vectors pseudotyped with the ***RD114*** envelope protein could be useful for achieving clinically relevant levels of gene transfer into human pluripotent hematopoietic cells.

L5 ANSWER 8 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322016 BIOSIS
DN PREV200100322016
TI Comparison of three retroviral envelopes for high efficiency gene transfer into human marrow mesenchymal cells.
AU Hofmann, Ted J. (1); Capizzani, Tony R. (1); Kelly, Patrick F. (1); Vanin, Elio F. (1); Horwitz, Edwin M. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 220a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB Bone marrow stromal cell (MSCs) are marrow mesenchymal cells that are ideal vehicles for delivery of therapeutic proteins in gene therapy protocols. A major obstacle to any successful gene therapy strategy is obtaining high efficiency transduction of the target cells. To optimize transduction of MSCs for clinical trials, we compared the effect of the retroviral envelope on gene transfer efficiency. Three different pseudotypes of a murine ***stem*** ***cell*** viral vector, encoding the green fluorescent protein (GFP) as a marker, were produced: amphotropic (Ampho) in PA317 cells, GALV in PG13 cells, and ***RD114*** (RD) in ***FLYRD18*** cells. The titer of each supernatant was determined using HeLa cells: Ampho = 4.1 X 104, GALV1 = 3.4 X 103, GALV2 = 1.2 X 105, and RD = 5.0 X 105 tu/ml. Following a standard 3-day transduction protocol, the human MSCs were analyzed by flow cytometry to determine the percentage of GFP positive cells. First, MSCs were transduced with Ampho (MOI = 0.2) yielding 92%; GALV1 (MOI = 0.02), 46%; GALV2 (MOI = 0.6), 68%; and RD (MOI = 2.5), 86% gene transfer. Next, MSCs were transduced with RD at an MOI of 0.2 (equivalent to Ampho) and 83% gene transfer was observed, not significantly different from the 86% transduction obtained using undiluted RD or the 92% obtained with Ampho. Finally, MSCs were transduced with either Ampho or RD at an MOI of 0.02 (equivalent to GALV1). Ampho transduced 77% and RD 61% of the MSCs, compared to 46% for GALV1. Notably, dilute RD (61%) and dilute Ampho (77%) transduced MSCs as well as the higher titer GALV2 (68%). Northern blot analysis showed an unexpected ratio (8:4:1) for the mRNAs of RDR (***RD114*** receptor), Pit-1 (GALV receptor), and Pit-2 (amphotropic receptor). Although RD and Ampho have similar potential to mediate gene transfer into MSCs, the mRNA for RDR is 8-fold more abundant than Pit-2 mRNA. Further, Pit-1 is 4-fold more abundant than Pit-2 despite the apparent lower gene transfer efficiency. We then compared the standard transduction of MSCs to transduction using RetroNectin coated dishes and found no difference in gene transfer efficiency. We conclude that amphotropic and ***RD114*** pseudotyped vectors are more effective for mediating gene transfer into MSCs. Further, more abundant receptor mRNA does not necessarily indicate a greater potential for transduction by the respective viral pseudotype. A higher titer GALV pseudotyped vector may be adequate for efficient transduction but sufficiently high titer PG13 supernatant has been difficult to generate. Additionally, RetroNectin does not enhance gene transfer in our system. Thus, ***RD114*** or amphotropic envelopes are preferred for clinical trials of MSC gene therapy.

L5 ANSWER 9 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322005 BIOSIS
DN PREV200100322005
TI Sustained multilineage gene persistence and expression in dogs transplanted with CD34+ marrow cells transduced by ***RD114*** pseudotyped oncoretroviral vectors.
AU Horn, Peter A. (1); Goerner, Martin (1); Peterson, Laura (1); Storb, Rainer (1); Kiem, Hans-Peter (1)
CS (1) Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 218a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB We have recently reported efficient gene transfer into canine hematopoietic repopulating cells using oncoretroviral vectors pseudotyped by the feline endogenous retrovirus envelope protein (***RD114***). Using a competitive repopulating assay in the dog model we compared gene transfer into hematopoietic ***stem*** ***cells*** between vectors produced by PG13 (GALV pseudotype) and FLYRD (***RD114*** pseudotype). CD34-enriched marrow cells from five dogs were divided into equal aliquots and transduced with LgGLSN (FLYRD), LNX (FLYRD) and LNY (PG13). All three vectors carried the neo gene and short sequence differences that allowed them to be distinguished in a single polymerase chain reaction. The ***RD114*** pseudotyped LgGLSN vector also contained the green fluorescent protein (GFP), enabling us to follow gene expression in transduced cells by flow cytometry. One animal died due to infection before sustained engraftment could be achieved and in the animal with lowest overall transduction rate follow-up was discontinued. We now present follow-up data of three dogs at 9, 18 and 21 months. Up to 10% of peripheral blood cells expressed GFP shortly after transplantation and up to 8% GFP-expressing cells were detected after 21 months. Flow cytometric analysis of hematopoietic subpopulations showed sustained GFP expression in all three dogs in DM5+ granulocytes, CD3+ lymphocytes and CD14+ monocytes. The percentage of GFP expressing cells was higher in granulocytes (up to 8.1%) than in lymphocytes (up to 3.5%) or monocytes (up to 5.7%). Two animals were examined for GFP expression in platelets and were found to have between 1.2-1.3% GFP+ platelets at 9 and at 21 months posttransplant. Since transduction efficiency has been shown to correlate with the level of retroviral receptor expression on target cells, we analyzed expression levels of the ***RD114*** receptor (RDR) on human and dog cells. Northern blot analysis revealed an almost 2-fold higher expression of RDR on human cells suggesting that human cells might be even more susceptible to transduction by ***RD114*** pseudotyped vectors than dog cells. In summary, our data show efficient transduction of canine hematopoietic repopulating cells using ***RD114*** pseudotyped retroviral vectors. The level of gene transfer and the sustained multilineage gene persistence and expression obtained in these experiments suggests that the ***RD114*** pseudotype is a promising alternative pseudotype for human ***stem*** ***cell*** gene therapy.

=> s retrovir? and adhere? and retronectin
L6 0 RETROVIR? AND ADHERE? AND RETRONECTIN

=> s retronectin
L7 23 RETRONECTIN

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 18 DUP REM L7 (5 DUPLICATES REMOVED)

=> d bib abs l8

L8 ANSWER 1 OF 18 CAPLUS COPYRIGHT 2002 ACS
AN 2001:678635 CAPLUS
DN 135:236393
TI Highly efficient gene transfer into human repopulating stem cells by RD114 envelope protein pseudotyped retroviral vector particles which pre-adsorb on ***retronectin*** -coated plates
IN Kelly, Patrick F.; Vanin, Elio F.
PA St. Jude Children's Research Hospital, USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001068150	A2	20010913	WO 2001-US7212	20010307

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2001051375	A1	20011213	US 2001-801302	20010307

PRAI US 2000-187534 P 20000307
AB The present invention relates to a method for efficiently introducing exogenous genes into stem cells, particularly human stem cells. The method optionally includes the steps of inducing the proliferation of target cells by pre-stimulation with cytokines and/or growth factors, followed by incubating these cells with RD114-pseudotyped vector particles. In a specific embodiment, the vector particles are

retronectin -immobilized or ultracentrifugation-concd. retroviral vector particles pseudotyped with the feline endogenous retrovirus (RD114) envelope protein. The present invention further discloses a method for somatic gene therapy, which can be used for various therapeutic applications and involves introducing a gene of interest contained within the retroviral genome into human repopulating stem cells followed by introducing these cells into a human host. Finally, the present invention discloses a method for monitoring the efficiency of the stem cell-mediated gene transfer based on detecting the presence of the genes (or the expression products) of the retroviral vector in various stem cell-derived lineages of the host.

=> d bib abs 2-
YOU HAVE REQUESTED DATA FROM 17 ANSWERS - CONTINUE? Y/(N):y

L8 ANSWER 2 OF 18 CAPLUS COPYRIGHT 2002 ACS
AN 2001:168163 CAPLUS
DN 134:203423
TI Improved transduction of pluripotent hematopoietic stem cells using retroviral gene delivery system, and use of retroviral particles in treatment of various disorders
IN Verstegen, Monique Maria Andrea; Wognum, Albertus Wernerus; Wagemaker, Gerard
PA Erasmus Universiteit Rotterdam, Neth.
SO PCT Int. Appl., 26 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001016341	A1	20010308	WO 2000-NL611	20000901
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1081227	A1	20010307	EP 1999-202859	19990902
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI EP 1999-202859	A	19990902		
EP 1999-203875	A	19991119		

AB The invention provides the materials and methods for improved transduction of CD34+ cells, from bone marrow or umbilical cord blood (UCB), using gene delivery vehicles of retroviral origin (retroviral particles). The invention relates that the CD34+ cells are cultured in the presence of fibronectin or ***retronectin*** . The invention also provides for use of transduced CD34+ cells in the expression of a heterologous protein when introduced into mammalian hosts. The invention further provides a method for prodn. of said gene delivery vehicles (retroviral particles). Finally, the invention provides: (1) pharmaceutical comprns. comprising said retroviral particles, and (2) use of said comprns. in treatment of a hereditary disorder or a pathol. condition related to a genetic aberration, and/or in prepn. of medicament for treatment of various disorders. The invention discussed that useful nucleic acid mols. can be provided to stem cells using the material and methods provided. The invention also discussed that an important variable in the efficiency of transduction is the ratio between the no. of cells and no. of transducing particles. The invention utilized the above improved method to transduce CD34+ human UCB cells, and human and rhesus monkey bone marrow cells with a retrovirus carrying the EGFP (enhanced green fluorescent protein) gene. The transduced cells were then transplanted into irradiated mice or rhesus monkeys and the expression of EGFP in bone marrow was detd.
RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:264678 BIOSIS
DN PREV200100264678
TI Cancer immunotherapy by genetically engineered effector lymphocytes redirected by chimeric receptors.
AU Eshhar, Zelig (1); Pinthus, Jehonathan H. (1); Waks, Tova (1); Bendavid, Alain (1); Schindler, Daniel G. (1)
CS (1) Weizmann Institute of Science, Rehovot, 76100 Israel
SO FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A1200. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
DT Conference
LA English
SL English
AB To expand the recognition spectrum of effector lymphocytes and redirect them to predefined targets, notably cancer cells, we endowed T and NK cells with antibody-type specificity, using chimeric receptor genes. Several configurations of chimeric receptors have been designed, mostly employing the anti-tumor antibody V region in the form of single chain variable fragment (scFv) as the recognition domain. As another recognition unit, we have replaced the extracellular scFv with the Neuregulin/NDF ligand, which binds to human adenocarcinoma cells over-expressing members of the erb-B onco-receptor family. To avoid anergy and antigen induced cell death, we have included the co-stimulatory CD28 molecule as part of the chimeric receptor and found that such a tri-partite receptor, containing scFv linked to CD28 as spacer and co-stimulatory moiety and the FcR g as stimulatory domain can indeed serve to fully activate resting T cells of transgenic mice harboring such chimeric receptor. To determine and optimize the clinical applicability of the chimeric receptor approach we have used an efficient procedure for the transduction of CD3/CD28 activated human T cells, employing retrovectors expressing GaLV envelopes and ***RetroNectin*** , a routine expression the chimeric receptors can be achieved in 40-70% of the cells. As a model, we have established a few human prostate cancer xenografts in SCID mice and demonstrated that local administration of human T cells expressing an HER/2-specific chimeric receptor could cause a complete rection of the tumors. We believe that prostate cancer is an excellent candidate for the chimeric receptor gene-immunotherapy not only because direct, intratumoral application of the genetically engineered lymphocytes is possible and because the metastatic pattern of prostate tumor (bones, lymph nodes) is readily accessible to T cells, but also because 'biological prostatectomy' is acceptable.

L8 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
AN 2001:549786 CAPLUS
DN 135:286233
TI The impact of ex vivo cytokine stimulation on engraftment of primitive hematopoietic cells in a non-human primate model
AU Dunbar, Cynthia E.; Takatoku, Masaaki; Donahue, Robert E.
CS Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, 20892, USA
SO Ann. N. Y. Acad. Sci. (2001), 938(Hematopoietic Stem Cells 2000), 236-245
CODEN: ANYAA9; ISSN: 0077-8923
PB New York Academy of Sciences
DT Journal
LA English
AB The impairment of engraftment ability after ex vivo or in vivo stimulation of hematopoietic stem cells, potentially related to induction of active cell cycling, has recently been a topic of intense interest. The authors' group has used the non-human primate autologous transplantation model and genetic marking to investigate a no. of questions in hematopoiesis with direct relevance to human clin. applications. The issue of a potential reversible engraftment defect would have many implications for gene therapy and allogeneic or autologous transplantation. Initial in vitro studies with rhesus CD34+ cells indicated that after 4 days of stimulatory culture in stem cell factor (SCF), megakaryocyte growth and development factor (MDGF), and flt3 ligand (FLT), transfer of the cells to SCF alone on ***retronectin*** (FN) support resulted in decreased active cycling and a halt to proliferation, without a loss of viability or induction of apoptosis. The authors then directly compared the engraftment potential of cytokine-stimulated cells vs. those transferred to SCF on FN alone before reinfusion. SCF/G-CSF mobilized CD34 cells from three animals were split into two parts and transduced with either of two retroviral marking vectors for 4 days in the presence of SCF/FLT/MGDF on FN. One aliquot was cryopreserved, and the other was continued in culture without transduction for 2 days in the presence of SCF alone on FN. After total body irradn., both aliquots were thawed and reinfused into each animal. In all animals, the level of marking from the fraction continued in culture for 2 days with SCF on FN was significantly higher than the level of marking from the aliquot transduced for 4 days without the 2-day period in SCF alone. This approach may allow more efficient engraftment of successfully transduced or ex vivo expanded cells by avoiding active cell cycling at the time of reinfusion.
RE.CNT 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2000:294355 BIOSIS
DN PREV200000294355
TI Efficient transduction of human hematopoietic repopulating cells generating stable engraftment of transgene-expressing cells in NOD/SCID mice.
AU Barquinero, Jordi; Segovia, Jose Carlos; Ramirez, Manuel; Limon, Ana; Guenechea, Guillermo; Puig, Teresa; Briones, Javier; Garcia, Juan; Bueren, Juan Antonio (1)
CS (1) Department of Molecular and Cellular Biology, CIEMAT, Madrid Spain
SO Blood, (May 15, 2000) Vol. 95, No. 10, pp. 3085-3093. print.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB In an attempt to develop efficient procedures of human hematopoietic gene therapy, retrovirally transduced CD34+ cord blood cells were transplanted into NOD/SCID mice to evaluate the repopulating potential of transduced grafts. Samples were prestimulated on ***Retronectin*** -coated dishes and infected with gibbon ape leukemia virus (GALV)-pseudotyped FMEV vectors encoding the enhanced green fluorescent protein (EGFP). Periodic analyses of bone marrow (BM) from transplanted recipients revealed a sustained engraftment of human hematopoietic cells expressing the EGFP transgene. On average, 33.6% of human CD45+ cells expressed the transgene 90 to120 days after transplantation. Moreover, 11.9% of total NOD/SCID BM consisted of human CD45+ cells expressing the EGFP transgene at this time. The transplantation of purified EGFP+ cells increased the proportion of CD45+ cells positive for EGFP expression to 57.7% at 90 to 120 days after transplantation. At this time, 18.9% and 4.3% of NOD/SCID BM consisted of CD45+/EGFP+ and CD34+/EGFP+ cells, respectively. Interestingly, the transplantation of EGFP- cells purified at 24 hours after infection also generated a significant engraftment of CD45+/EGFP+ and CD34+/EGFP+ cells, suggesting that a number of transduced repopulating cells did not express the transgene at that time. Molecular analysis of NOD/SCID BM confirmed the high levels of engraftment of human transduced cells deduced from FACS analysis. Finally, the analysis of the provirus insertion sites by conventional Southern blotting indicated that the human hematopoiesis in the NOD/SCID BM was predominantly oligoclonal.

L8 ANSWER 6 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:317226 BIOSIS
DN PREV200100317226
TI Storage of factor VIII (FVIII) in the alpha-granules of human platelets following retroviral transduction and transplantation of human CD34+ cells into NOD-SCID mice.
AU Wilcox, David A. (1); Rosenberg, Jonathan B.; Johnson, Bryon D. (1); Montgomery, Robert R. (1)
CS (1) Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 803a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB In order to develop methods for gene therapy of disorders affecting hemostasis, we transduced Isolex(R) selected CD34+ cells (Nexell Therapeutics) from human mobilized peripheral blood with a retroviral vector encoding human FVIII (Chiron Technologies). CD34+ cells were transduced on plates coated with ***RetroNectin*** (Takara Shuzo) in the presence of SCF, flt-3/flk-2 ligand, IL-6, and pegylated recombinant human Megakaryocyte Growth and Differentiation Factor (Kirin Brewery).

Indirect immunofluorescence analysis using antibodies against human FVIII, vWF, and the megakaryocyte-specific marker, glycoproteins (GP) IIb-IIIa revealed that megakaryocytes derived from transduced CD34+ cells in vitro could synthesize FVIII and traffick it to alpha-granules in association with von Willebrand factor (vWF). This result was similar to trafficking previously observed for these molecules to Weibel-Palade bodies in FVIII-transduced endothelial cells. FVIII was also detected in the cytoplasm of cultured cells that were negative for vWF or GPIIb-IIIa staining, indicating that transduction was not limited to the megakaryocyte lineage. To examine the effect of FVIII expression in platelets, in vivo, FVIII-transduced CD34+ cells were transplanted into NOD-SCID mice treated with a sublethal dose (350 cGy) of irradiation. Flow cytometric analysis using antibodies specific for human GPIIb-IIIa revealed that circulating human platelets comprised up to 40% of the total platelet population in whole blood isolated from the mice during 2-6 weeks post-transplant. Immunofluorescence analysis using confocal microscopy revealed a punctuate staining for FVIII that was colocalized with vWF to alpha-granules in a subpopulation of human platelets isolated from murine whole blood. In contrast, FVIII was not detected in murine platelets. These results indicate that human megakaryocytes can synthesize and store FVIII with vWF in alpha-granules that can be retained in progeny platelets. We speculate that FVIII could undergo regulated release from platelets following physiologic hemostatic response to vessel injury. This raises the possibility of developing a locally inducible secretory pool of FVIII in platelets of patients with hemophilia A following autologous transplantation of FVIII-transduced CD34+ peripheral blood cells.

L8 ANSWER 7 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322415 BIOSIS
DN PREV200100322415
TI Ex vivo expansion of primitive hematopoietic cells by reduction of p21cip1/waf1 expression level.
AU Stier, S. (1); Cheng, T. (1); Miura, N. (1); Dombkowski, D. (1); Sarmento, L. M. (1); Scadden, D. T. (1)
CS (1) Exp. Hematology, Massachusetts General Hospital, Charlestown, MA USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 667a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The quiescence of hematopoietic stem cells is critical to prevent the exhaustion of the hematopoietic system in vivo, while limiting the clinical applicability of ex vivo stem cell expansion and gene therapy. Current protocols for ex vivo expansion of stem cells involve the use of differentiation inducing cytokines, which often leads to a decreased multipotentiality of the expanded cell pool. Implicated in the maintenance of stem cell quiescence is the CDK inhibitor p21cip1/waf1 (p21) (Science 287,2000:1804). p21-knock out mice showed an increase of absolute hematopoietic stem cell number under normal homeostatic conditions and premature death due to hematopoietic cell depletion after cell cycle specific myelotoxic injury in comparison to widtype mice. These findings suggest an alternative strategy of ex vivo stem cell expansion maintaining the multipotentiality of stem cells by altering the p21 expression levels. Therefore, we transduced CD34+ and CD34+38- cord blood cells with a VSV-G pseudotyped lentiviral vector containing full length p21-antisense (p21-AS). After transduction for 20 hrs on two successive days in the presence of KL(50ng/ml), Flt-3-L(50ng/ml), TPO(25ng/ml), IL-3(10ng/ml) and polybrene(4mug/ml) on ***retronectin*** coated wells a transduction efficiency of 45-55% for the control vector and 25-35% for the p21-AS vector could be observed. The p21-AS transduced CD34+ and CD34+38- cells showed a 3.4- and 2.7-fold increase in the CFU-mix colony number in comparison to the control vector transduced cells (CD34+: 9.3 vs. 2.7 col. per 600 cells, p=0.016; CD34+38-: 19.2 vs. 7.1 col. per 600 cells, p=0.013), whereas the total colony number was not significantly increased. The stem cell number present in the transduced cell population was directly measured by limit-dilution LTC-IC assays. A significant increase in primitive cells in the p21-AS transduced CD34+ and CD34+38- cells in comparison to the control vector transduced cells was noted (CD34+: 33.5 vs. 19.3 LTC-ICs per 105 cells, p=0.037; CD34+38-: 205.6 vs. 82.4 LTC-ICs per 105 cells). Furthermore, 8 weeks after transplantation into sublethal irradiated NOD/SCID mice p21-AS transduced CD34+ cells showed a 20-fold higher repopulating potential than control vector tranduced cells. These results demonstrate a specific expansion of primitive cells in hematopoietic cell pools by reduction of p21 expression. Therefore, reducing p21 expression level offers a new approach for ex vivo hematopoietic stem cell expansion.

L8 ANSWER 8 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322183 BIOSIS
DN PREV200100322183
TI Comparative analysis of gene marking and lineage development in SCID-repopulating cells derived from cord blood or mobilized peripheral blood.
AU Pollok, Karen E.; van der Loo, Johannes C. M.; Cooper, Ryan J.; Hartwell, Jennifer R.; Miles, Katherine R.; Breese, Robert; Williams, David A.
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 589a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB Efficient transfer and expression of therapeutic genes in long-term repopulating cells derived from G-CSF-mobilized peripheral blood CD34+ cells (MPB) is a priority for many clinical gene therapy protocols. The efficiency of gene transfer in MPB SCID-repopulating cells (SRCs) was compared to gene transfer in SRCs derived umbilical cord blood CD34+ cells (CB). Pre-stimulated CB or MPB cells were infected twice on FN CH-296 (***Retronectin*** (R), Takara Shuzo) utilizing a GALV-pseudotyped MFG-EGFP retroviral vector at an identical multiplicity of infection (MOI = 2) and transplanted into NOD/SCID mice. Flow cytometric analysis and clonogenic assays indicated that approximately 70% of the input CB cells were EGFP+, while 35-50% of input MPB cells were EGFP+. This discrepancy was even more striking in SRCs derived from CB versus those derived from MPB. At 8-9 weeks post-transplant, 35-40% of the CB-derived human cells repopulating NOD/SCID mice in bone marrow (BM) and spleen (n=11) were EGFP+, while in MPB transplant recipients, human cells in BM and spleen were only 0.4-4.0% EGFP+ (n=23). Low levels of gene marking in MPB were confirmed by PCR of individual human colonies from the BM. In recipients of both CB and MPB, immature B-cell progenitors (CD34+, CD19+), mature B cells (CD34-, CD19+) and myeloid (CD45+, CD33+) lineages contained gene-marked cells. SRCs in MPB may require a longer pre-stimulation time for entry into cell cycle. Therefore, MPB (n=41) was transduced after 4-8 days of pre-stimulation. Although human cell engraftment was observed under all pre-stimulation conditions, gene-transfer levels in both lymphoid and myeloid lineages ranged from 0.5-8.0% for MPB. An exception was noted in one MPB donor in which gene transfer following a 6-day pre-stimulation period resulted in 6-16% EGFP+ human cells in the BM. PKH2 staining of MPB was employed to evaluate proliferation following pre-stimulation. After 6-8 days of ex vivo expansion followed by transduction, approximately 1-2.0% of the MPB was PKH2+, EGFP- indicative of a small population of cells that was still refractive to stimulation and transduction (n=5). Long-term repopulating cells still existed in MPB ex vivo expanded for up to 10 days, since human cells were detected by genomic Southern in the bone marrow of secondary NOD/SCID transplants. In conclusion, a significant discrepancy exists in the ability to effectively introduce genes into SRCs derived from MPB as compared to CB. Strategies utilizing in vivo selection or alternative vector systems may be necessary to achieve high levels of transduced MPB SRCs.

L8 ANSWER 9 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302193 BIOSIS
DN PREV200100302193
TI Multilineage transduction of non-human primate CD34+ hematopoietic cells using RD-114 pseudotyped oncoretroviruses.
AU Kelly, Patrick F. (1); Bonifacino, Aylin C.; Carrington, Jody A. (1); Agricola, Brian A.; Metzger, Mark E.; Kluge, Kim A.; Nienhuis, Arthur W. (1); Donahue, Robert E.; Vanin, Elio F. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 525a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The relative quiescence of the hematopoietic stem cell (HSC) and the low level of viral receptor expression are known to contribute to the low efficiency of retroviral gene transfer into HSCs of large animals and humans. We have previously reported that RD114-pseudotyped retroviruses could efficiently transduce cord blood CD34+ cells after 24-48 hours pre-stimulation and a single exposure to the viral particles preloaded onto ***RetroNectin*** -coated plates. Based on these results we evaluated gene transfer of RD114-pseudotyped murine retroviruses using non-human primate CD34+ peripheral blood (PB) cells in the rhesus autologous transplant model. SCF/G-CSF-mobilized rhesus monkey PB were collected and enriched for CD34+ cells. These cells were cultured in serum-containing medium with high concentrations of SCF, FLT-3 and IL-6 and exposed to RD114-pseudotyped particles preloaded onto ***RetroNectin*** -coated plates at 48 hours and 72 hours. After 96 hours in culture, cells were harvested and infused into irradiated recipients (2 X 500 cGy, n=5). The transduction efficiency of the infused cells was 35-55% based on EGFP expression. In all animals we have observed multilineage engraftment with persistence of EGFP expression after 6-8 weeks post-transplantation, a result that was not achieved with a similar construct pseudotyped with the amphotropic envelope protein. In the first animal transplanted, we observed high levels of multilineage engraftment of EGFP+ cells (as high as 98% in granulocytes) over the first 20 weeks post transplantation. After 26 weeks multilineage expression has stabilized at 8-10%. Serial genomic southern analysis for both proviral integrity and integration site indicated that vector silencing was not occurring and that the engraftment of gene modified cells was oligoclonal. The second recipient displayed similar kinetics but died from transplant related complications 8 weeks post-transplantation. Subsequent animals have achieved lower levels of EGFP expression (1-3%) suggesting that transduction conditions using this pseudotype remains to be optimized. These results suggest oncoretroviral vectors pseudotyped with the RD114 envelope protein could be useful for achieving clinically relevant levels of gene transfer into human pluripotent hematopoietic cells.

L8 ANSWER 10 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302190 BIOSIS
DN PREV200100302190
TI In vivo expansion of gene-modified hematopoietic cells by the selective amplifier gene in a nonhuman primate model.
AU Hanazono, Yutaka (1); Nagashima, Takeyuki; Shibata, Hiroaki; Ageyama, Naohide; Asano, Takayuki (1); Ueda, Yasuji; Kume, Akihiro (1); Terao, Keiji; Hasegawa, Mamoru; Ozawa, Keiya (1)
CS (1) Div. Genet. Therapeut., Jichi Med. Sch., Tochigi Japan
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 524a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB Although hematopoietic stem cells (HSCs) have been pursued as desirable targets for gene therapy, clinical studies indicate that the gene transfer efficiency into human HSCs is too low to be of clinical utility in most situations. To overcome this problem, we developed a method of in vivo expansion of transduced cells. In this system, target cells are harnessed with the selective amplifier gene (SAG), a chimeric gene of the G-CSF receptor and the estrogen receptor hormone-binding domain. We deleted the G-CSF-binding domain from the chimeric gene to abolish the responsiveness to G-CSF and introduced a mutation (Y703F) to prevent the differentiation signal transduction. We demonstrated that the SAG product predominantly transmits the proliferation signal with the minimal differentiation signal in response to estrogen in vitro. We then examined the in vivo effect of the SAG in a cynomolgus macaque model. Cynomolgus bone marrow CD34+ cells were transduced with MSCV-based, GALV-pseudotyped retroviral vectors with or without the SAG (n=3). The supernatant transduction was performed for 4 days with ***retronectin*** (supplied by Takara) and cytokines including Flt-3 ligand. The transduced cells were reinfused into each myeloablated monkey (500cGy X 2). After transplantation, bone marrow cells were taken and each colony formed by the cells was subjected to PCR in search of the provirus. In two monkeys without the SAG, around 10% of colony-forming progenitors contained the provirus for 1 year

posttransplant. In the other monkey (female) with the SAG, although only 10% of progenitors contained the provirus before reinfusion, the provirus was detected in approximately 40% of progenitors posttransplant even without administration of estrogen. Some progenitors with the SAG responded to the endogenous estrogen. Since the proportion of the provirus-containing progenitors dropped to 5% 6 months posttransplant, estradiol was administered to the monkey. The progenitors with the provirus then increased to 30% in response to the exogenous estrogen. These results suggest that, with inclusion of the SAG in retroviral vectors, gene modified hematopoietic progenitors could be selectively expanded in vivo by treatment with estrogen.

L8 ANSWER 11 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322016 BIOSIS
DN PREV200100322016
TI Comparison of three retroviral envelopes for high efficiency gene transfer into human marrow mesenchymal cells.
AU Hofmann, Ted J. (1); Capizzani, Tony R. (1); Kelly, Patrick F. (1); Vanin, Elio F. (1); Horwitz, Edwin M. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 220a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB Bone marrow stromal cell (MSCs) are marrow mesenchymal cells that are ideal vehicles for delivery of therapeutic proteins in gene therapy protocols. A major obstacle to any successful gene therapy strategy is obtaining high efficiency transduction of the target cells. To optimize transduction of MSCs for clinical trials, we compared the effect of the retroviral envelope on gene transfer efficiency. Three different pseudotypes of a murine stem cell viral vector, encoding the green fluorescent protein (GFP) as a marker, were produced: amphotropic (Ampho) in PA317 cells, GALV in PG13 cells, and RD114 (RD) in FLYRD18 cells. The titer of each supernatant was determined using HeLa cells: Ampho = 4.1 X 104, GALV1 = 3.4 X 103, GALV2 = 1.2 X 105, and RD = 5.0 X 105 tu/ml. Following a standard 3-day transduction protocol, the human MSCs were analyzed by flow cytometry to determine the percentage of GFP positive cells. First, MSCs were transduced with Ampho (MOI = 0.2) yielding 92%; GALV1 (MOI = 0.02), 46%; GALV2 (MOI = 0.6), 68%; and RD (MOI = 2.5), 86% gene transfer. Next, MSCs were transduced with RD at an MOI of 0.2 (equivalent to Ampho) and 83% gene transfer was observed, not significantly different from the 86% transduction obtained using undiluted RD or the 92% obtained with Ampho. Finally, MSCs were transduced with either Ampho or RD at an MOI of 0.02 (equivalent to GALV1). Ampho transduced 77% and RD 61% of the MSCs, compared to 46% for GALV1. Notably, dilute RD (61%) and dilute Ampho (77%) transduced MSCs as well as the higher titer GALV2 (68%). Northern blot analysis showed an unexpected ratio (8:4:1) for the mRNAs of RDR (RD114 receptor), Pit-1 (GALV receptor), and Pit-2 (amphotropic receptor). Although RD and Ampho have similar potential to mediate gene transfer into MSCs, the mRNA for RDR is 8-fold more abundant than Pit-2 mRNA. Further, Pit-1 is 4-fold more abundant than Pit-2 despite the apparent lower gene transfer efficiency. We then compared the standard transduction of MSCs to transduction using ***RetroNectin*** coated dishes and found no difference in gene transfer efficiency. We conclude that amphotropic and RD114 pseudotyped vectors are more effective for mediating gene transfer into MSCs. Further, more abundant receptor mRNA does not necessarily indicate a greater potential for transduction by the respective viral pseudotype. A higher titer GALV pseudotyped vector may be adequate for efficient transduction but sufficiently high titer PG13 supernatant has been difficult to generate. Additionally, ***RetroNectin*** does not enhance gene transfer in our system. Thus, RD114 or amphotropic envelopes are preferred for clinical trials of MSC gene therapy.

L8 ANSWER 12 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322004 BIOSIS
DN PREV200100322004
TI Highly efficient retroviral gene transfer to human cord blood CD34+/CD38low and NOD/SCID repopulating cells using a simplified transduction protocol.
AU Relander, Thomas (1); Karlsson, Stefan (1); Richter, Johan (1)
CS (1) Molecular Medicine and Gene Therapy, University Hospital, Lund Sweden
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 217a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB We investigated retroviral gene transfer to human cord blood CD34+/CD38+, CD34+/CD38low and NOD/SCID repopulating cells and compared transduction efficiency using an MSCV based vector with the gene for GFP (MGIN) which was packaged into 3 different cell lines: PG13 (GALV), 293GPG (VSV-G) or GP+env-AM12 (amphotropic). Viral titer was 1-3X106 inf. units/ml for PG13-MGIN and AM12-MGIN; for 293GPG-MGIN up to 107. Cord blood CD34+ cells were sorted into CD38 low (6% lowest) or CD38+ fractions to study kinetics of transduction and were cultured in serum-free medium with MGDF, FL and SCF (100 ng/ml) before transduction with a single 24 hour hit in ***Retronectin*** (RN) coated wells preloaded with vector on days 0-5. Efficient transduction of CD38+ cells was observed already after one day of pre-stimulation and then was at approximately the same level through day 4; 59-67% (PG13), 23-30% (293GPG) and 39-51 % (AM12) However, CD38low cells were not efficiently transduced until day 3 day but level of GFP+ cells was then approximately the same as for the CD38+ cells; 62%, 29 % and 39 %, respectively. In 3 NOD/SCID experiments, cells were cultured as above for 48 hrs before transduction (with serum (SC) or serum free (SF)) on RN pre-loaded with virus alone followed by addition of 1/10 volume of virus supernatant at 72 hrs without further manipulations. At 96 hrs cells were harvested and injected into irradiated NOD/SCID mice (250.000 EE/mouse), which were analyzed at 6 w. Compared to engraftment of fresh cells (44% SD 25,6) transduction under SC but not SF conditions resulted in significantly lower engraftment. All three envelopes tested efficiently transduced SRC but transduction measured by FACS and GFP+ CFU was significantly higher for PG13SF when compared to 293GPG and AM12. Transplantation of fresh and PG13SF transduced cells at limiting dilution showed no loss of engraftment capacity of transduced cells. Engraftment of GFP positive human cells with as low as 15.625EE was observed. Conclusions: Highly efficient retroviral transduction of primitive human hematopoietic progenitors without loss of repopulating activity can be achieved using a very simple protocol with RN preloaded with virus. The PG13 pseudotyped vector used under serum free conditions gave the best results.

L8 ANSWER 13 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:321993 BIOSIS
DN PREV200100321993
TI Fetal liver stromal cell line AFT024 enhances gene transfer in primitive human hematopoietic cells in mobilized peripheral blood.
AU Van Der Loo, Johannes C. M. (1); Eaton, Kristin S. (1)
CS (1) Medicine, University of Minnesota, Minneapolis, MN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 215a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB NOD/SCID transplant studies show that primitive hematopoietic cells in human G-CSF mobilized peripheral blood (MPB) are more difficult to transduce than cells from umbilical cord blood (UCB). We hypothesize that primitive hematopoietic cells in MPB are refractive to gene transfer (GT) due to insufficient cytokine stimulation prior to retroviral infection. Earlier studies have demonstrated a positive effect of the fetal liver stromal cell line AFT024 on the maintenance of primitive hematopoietic cells ex vivo in the presence of low doses of early acting cytokines. Based on these data we propose that AFT024 may enhance the level of GT in primitive hematopoietic cells in MPB. To test this hypothesis, CD34+ cells from MBP were cultured for four days in the presence or absence of irradiated AFT024 cells using trans-well (non-contact) cultures with either G-CSF, SCF and TPO (GST; 100 ng/mL) or Flt3-L, SCF, IL-7 and TPO (FS7T; 10-20 ng/mL), followed by infection with a GALV-pseudotyped MFG-EGFP retroviral vector on ***Retronectin*** (R) (Takara Shuzo) on two consecutive days (m.o.i. = 2). The level of GT as well as the level of expansion was quantified using CFC and LTC-IC assays. AFT024 had a positive effect on the expansion of both CFC and LTC-IC (both 2-fold increase) independent of the cytokines used. In the presence of AFT024, the level of GT in CFC (ranging from 1 to 26% in BFU-E and CFU-GM; n = 10) was higher in the groups pre-stimulated with GST, while the level of GT in LTC-IC (ranging from 1.5 to 47%) was higher with FS7T (n=6). Overall, the recovery of transduced LTC-IC was 5 to 8-fold higher in the presence of AFT024 with FS7T as compared to our previously used strategy using GST in the absence of stroma (p<0.001). As the expansion and, therefore, the cell cycle behavior of CFC and LTC-IC was similar, these data indicate that the growth factors used have an additional but differential effect on the level of GT in primitive and less primitive cells. Finally, for application in clinical protocols, we demonstrate that AFT024 cells can be replaced by AFT024-conditioned medium without loss of transduced LTC-IC (n=5, ns). In conclusion, we demonstrate that the recovery of transduced primitive hematopoietic cells in G-CSF MPB can be enhanced using low doses of early acting cytokines and (a) soluble factor(s) produced by the cell line AFT024.

L8 ANSWER 14 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:321986 BIOSIS
DN PREV200100321986
TI Lentiviral vectors effectively transfer and express human glucose 6-phosphate dehydrogenase (G6PD) in primitive human hematopoietic cells (HSC) engrafting NOD/SCID mice.
AU Notaro, Rosario (1); Levy, Carolyn Fein (1); De Angioletti, Maria (1); Vanegas, Olga Camacho (1); Rovira, Ana (1); Sadelain, Michel (1); Luzatto, Lucio (1)
CS (1) Human Genetics, MSKCC, New York, NY USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 213a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB Lentiviral (LV) vectors, based on HIV, are emerging as powerful tools for transducing HSC. However, comparative data on LV vectors versus conventional murine leukemia virus (MLV) vectors with respect to optimizing transduction conditions and measuring transduction efficiency have been scarce. We have previously, transferred and expressed hG6PD in bona fide HSC using MLV vectors pseudotyped with the vesicular stomatitis virus G glycoprotein (VSVG). We have now constructed a VSVG-pseudotyped LV vector in which the hG6PD cDNA is under the transcriptional control of the CMV promoter. This LV vector was used to transduce lineage negative cord blood cells in serum-free medium (MOI apprx25) on ***retronectin*** coated plates. We tested various transduction conditions: (1) 5 hrs with or without cytokines; (2) 12 hrs of pre-culture followed by one or more transduction cycles of 12 hrs with cytokines. The transduced cells were (a) plated for hematopoietic colony forming cells (CFC) and (b) injected into sub-lethally irradiated NOD/SCID mice. In most of the expressing CFC the level of the transferred G6PD was at least as much as that of the endogenous G6PD. The LV vector was able to transfer and express 6GPD in a significant proportion of committed progenitors under all transduction conditions. However, in order to obtain expression in primitive HSC, 12 hours of pre-culture time and the use of cytokines were needed. In conclusion, primitive human HSC that are able to engraft into NOD/SCID mice need "priming" to be effectively transduced by LV vectors; transduction efficiency with LV vectors (apprx40%) is higher than that we have previously obtained with MLV vectors (apprx20%) using a MOI of apprx100. A definitive comparison between LV and MLV vectors under identical transduction conditions is needed.

L8 ANSWER 15 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:385012 BIOSIS
DN PREV200000385012
TI Centrifugation-enhanced retroviral gene transduction of human CD34+ cells in RetroNectinTM-coated gas permeable X-FoldTM containers.
AU Thornton, J. (1); Goel, A.; Tseng-Law, J.; Szalay, P.; Malech, H.; Van Epps, D.; Freimark, B.
CS (1) Nexell Therapeutics Inc., Irvine, CA USA
SO Experimental Hematology (Charlottesville), (July, 2000) Vol. 28, No. 7 Supplement 1, pp. 125. print.

Meeting Info.: 29th Annual Meeting of the International Society for Experimental Hematology Tampa, Florida, USA July 08-11, 2000 International Society for Experimental Hematology
. ISSN: 0301-472X.
DT Conference
LA English
SL English

L8 ANSWER 16 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 1999:397479 BIOSIS
DN PREV199900397479
TI Optimization of retroviral gene transduction of mobilized primitive hematopoietic progenitors by using thrombopoietin, Flt3, and Kit ligands and ***RetroNectin*** culture.
AU Murray, Lesley (1); Luens, Karin; Tushinski, Robert; Jin, Liang; Burton, Michelle; Chen, Jingyi; Forestell, Sean; Hill, Beth
CS (1) SyStemix, 3155 Porter Drive, Palo Alto, CA, 94304 USA
SO Human Gene Therapy, (July 20, 1999) Vol. 10, No. 11, pp. 1743-1752.
ISSN: 1043-0342.
DT Article
LA English
SL English
AB We have investigated the ability of several cytokine combinations to improve retrovirus-mediated transduction of human primitive hematopoietic progenitors (PHPs) from mobilized peripheral blood (MPB). Retroviral infection of CD34+ cells was performed by culture on fibronectin fragment CH-296 (***RetroNectin*** , RN), using the truncated human nerve growth factor receptor (NGFR) as the transgene reporter. Transgene expression among progeny of PHPs was assayed by FACS analysis after long-term stromal culture (LTC). Transgene delivery to PHPs was assessed by PCR of individual stromal culture-derived methylcellulose colonies (LTC-CFCs). Compared with interleukin 3 (IL-3), IL-6, and leukemia inhibitory factor (LIF), the combination of thrombopoietin (TPO), Flt3 ligand (FL), and Kit ligand (KL) effected a 73-fold increase in NGFR expression among CD34+ cells (to 14%) and a 14-fold increase in NGFR expression among total cells (to 10%) after LTC. In addition, a 2.4-fold increase in neo gene marking of LTC-CFCs was observed. A preclinical study comparing the effect of high-speed centrifugation ("spinoculation") or culture on RN during exposure to retroviral particles in teflon cell culture bags showed no difference in the efficiency of transduction of PHPs between these two methods.

L8 ANSWER 17 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:46646 BIOSIS
DN PREV200000046646
TI Immobilization of suspension cells on extracellular matrix: An on and off affair.
AU Prokopishyn, Nicole L. (1); Barron, Gina L. (1); Carsrud, N. D. Victor (1); Brown, David B. (1); Yannariello-Brown, Judith (1)
CS (1) Gene-Cell, Inc., Houston, TX USA
SO Blood, (Nov. 15) Vol. 94, No. 10 SUPPL. 1 PART 2, pp. 167b.
Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology New Orleans, Louisiana, USA December 3-7, 1999 The American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English

L8 ANSWER 18 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:113886 BIOSIS
DN PREV199900113886
TI Transduction kinetics of non-human primate immuno-selected CD34+ cells using retroviral and lentiviral vectors that express the green fluorescent protein.
AU Donahue, R. E. (1); Rowe, T. K.; Sorrentino, B. P.; Hawley, R. G.; An, D. S.; Chen, I. S. Y.; Wersto, R. P.
CS (1) Hematol. Branch, NHLBI, Rockville, MD USA
SO Blood, (Nov. 15, 1998) Vol. 92, No. 10 SUPPL. 1 PART 1-2, pp. 376B.
Meeting Info.: 40th Annual Meeting of the American Society of Hematology Miami Beach, Florida, USA December 4-8, 1998 The American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English

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(FILE 'HOME' ENTERED AT 11:08:13 ON 05 FEB 2002)

FILE 'BIOSIS, CAPLUS, EMBASE' ENTERED AT 11:08:30 ON 05 FEB 2002
L1 244 S RD114
L2 261 S L1 OR FLYRD18
L3 1 S L2 AND STEM CELL? AND LENTIVIR?
L4 15 S L2 AND STEM CELL?
L5 9 DUP REM L4 (6 DUPLICATES REMOVED)
L6 0 S RETROVIR? AND ADHERE? AND RETRONECTIN
L7 23 S RETRONECTIN
L8 18 DUP REM L7 (5 DUPLICATES REMOVED)

=> s transduc? and (adhere? or adsorb)
L9 3010 TRANSDUC? AND (ADHERE? OR ADSORB)

=> s l9 and retrovir
L10 0 L9 AND RETROVIR

=> s l9 and retrovir?
L11 136 L9 AND RETROVIR?

=> s l11 and py<1999
1 FILES SEARCHED...
L12 99 L11 AND PY<1999

=> s transduc? and (adhere? or adsorb?)
L13 3371 TRANSDUC? AND (ADHERE? OR ADSORB?)

=> s l13 and retrovir?
L14 136 L13 AND RETROVIR?

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1 FILES SEARCHED...
L15 99 L14 AND PY<1999

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PROCESSING COMPLETED FOR L15
L16 46 DUP REM L15 (53 DUPLICATES REMOVED)

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YOU HAVE REQUESTED DATA FROM 46 ANSWERS - CONTINUE? Y/(N):y

L16 ANSWER 1 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:374275 BIOSIS
DN PREV199800374275
TI ***Transduction*** of genes using ***retroviral*** vectors.
AU Spector, David L. (1); Goldman, Robert D.; Leinwand, Leslie A.
CS (1) Cold Spring Harbor Lab., New York, NY USA
SO Spector, D. L.; Goldman, R. D.; Leinwand, L. A.. (1998) pp. 92,1-92,20.
Cells: A Laboratory Manual, Vol. 2; Light microscopy and cell structure.
Publisher: Cold Spring Harbor Laboratory Press 10 Skyline Drive, Plainview, New York 11803, USA.
ISBN: 0-87969-521-8 (paper), 0-87969-522-6 (cloth).
DT Book
LA English

L16 ANSWER 2 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 1998:229121 BIOSIS
DN PREV199800229121
TI Evidence for keratinocyte stem cells in vitro: Long term engraftment and persistence of transgene expression from ***retrovirus*** - ***transduced*** keratinocytes.
AU Kolodka, Taduesz; Garlick, Jonathan A.; Taichman, Lorne B. (1)
CS (1) State Univ. New York at Stony Brook, Westchester Hall, Stony Brook, NY 11794-8702 USA
SO Proceedings of the National Academy of Sciences of the United States of America, (***April 14, 1998***) Vol. 95, No. 8, pp. 4356-4361.
ISSN: 0027-8424.
DT Article
LA English
AB Epidermis is renewed by a population of stem cells that have been defined in vivo by slow turnover, label retention, position in the epidermis, and enrichment in beta1-integrin, and in vitro by clonogenic growth, prolonged serial passage, and rapid ***adherence*** to extracellular matrix. The goal of this study is to determine whether clonogenic cells with long-term growth potential in vitro persist in vivo and give rise to a fully differentiated epidermis. Human keratinocytes were genetically labeled in culture by ***transduction*** with a ***retrovirus*** encoding the lacZ gene and grafted to athymic mice. Analysis of the cultures before grafting showed that 21.1-27.8% of clonogenic cells with the capacity for >30 generations were successfully ***transduced*** . In vivo, beta-galactosidase (beta-gal) positive cells participated in the formation of a fully differentiated epithelium and were detected throughout the 40-week postgraft period, initially as loosely scattered clusters and later as distinct vertical columns. Viable cells recovered from excised grafts were seeded at clonal densities and 23.3-33.3% of the colonies thus formed were beta-gal positive. In addition, no evidence of transgene inactivation was obtained: all keratinocyte colonies recovered from grafted tissue that were beta-gal negative also lacked the lacZ transgene. These results show that cells with long-term growth properties in vitro de indeed persist in vivo and form a fully differentiated epidermis, thereby exhibiting the properties of stem cells.

L16 ANSWER 3 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 1998:182088 BIOSIS
DN PREV199800182088
TI Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1.
AU Platt, Emily J.; Wehrly, Kathy; Kuhmann, Shawn E.; Chesebro, Bruce; Kabat, David (1)
CS (1) Dep. Biochemistry Molecular Biol., L224, 3181 SW Sam Jackson Park Rd., Portland, OR 97201-3098 USA
SO Journal of Virology, (***April, 1998***) Vol. 72, No. 4, pp. 2855-2864.
ISSN: 0022-538X.
DT Article
LA English
AB It has been proposed that changes in cell surface concentrations of coreceptors may control infections by human immunodeficiency virus type 1 (HIV-1), but the mechanisms of coreceptor function and the concentration dependencies of their activities are unknown. To study these issues and to generate stable clones of ***adherent*** cells able to efficiently titer diverse isolates of HIV-1, we generated two panels of HeLa-CD4/CCR5 cells in which individual clones express either large or small quantities of CD4 and distinct amounts of CCR5. The panels were made by ***transducing*** parental HeLa-CD4 cells with the ***retroviral*** vector SFF-CCR5. Derivative clones expressed a wide range of CCR5 quantities which were between 7.0 X 102 and 1.3 X 105 molecules/cell as measured by binding antibodies specific for CCR5 and the chemokine (125I)Mip1beta. CCR5 was mobile in the membranes, as indicated by antibody-induced patching. In cells with a large amount of CD4, an unexpectedly low trace of CCR5 (between 7 X 102 and 2.0 X 103 molecules/cell) was sufficient for maximal susceptibility to all tested HIV-1, including primary patient macrophagetropic and T-cell-tropic isolates. Indeed, the titers as indicated by immunoperoxidase staining of infected foci were as high as the tissue culture infectious doses measured in human peripheral blood mononuclear cells. In contrast, cells with a small amount of CD4 required a much larger quantity of CCR5 for maximal infection by macrophagetropic HIV-1 (ca. 1.0 X 104 to 2.0 X 104 molecules/cell). Cells that expressed low and high amounts of CD4 were infected with equal efficiencies when CCR5 concentrations were above threshold levels for maximal infection. Our results suggest that the concentrations of CD4 and CCR5 required for efficient infections by macrophagetropic HIV-1 are interdependent and that the requirements for each are increased when the other component is present in a limiting amount. We conclude that CD4 and CCR5 directly or indirectly interact in a concentration-dependent manner within a pathway that is essential for infection by macrophagetropic HIV-1. In addition, our results suggest that multivalent virus-receptor bonds and diffusion in the membrane contribute to HIV-1 infections.

L16 ANSWER 4 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3

AN 1998:166505 BIOSIS
DN PREV199800166505
TI ***Retroviral*** -mediated gene transfer of the leukocyte integrin CD18 into peripheral blood CD34+ cells derived from a patient with leukocyte adhesion deficiency type 1.
AU Bauer, Thomas R., Jr. (1); Schwartz, Barbara R.; Liles, W. Conrad; Ochs, Hans D.; Hickstein, Dennis D.
CS (1) VA Puget Sound Health Care System, GMR 151, 1660 S. Columbian Way, Seattle, WA 98108 USA
SO Blood, (***March 1, 1998***) Vol. 91, No. 5, pp. 1520-1526.
ISSN: 0006-4971.
DT Article
LA English
AB Leukocyte adhesion deficiency or LAD is a congenital immunodeficiency disease characterized by recurrent bacterial infections in which the leukocytes from affected children fail to ***adhere*** to endothelial cells and migrate to the site of infection due to heterogeneous defects in the leukocyte integrin CD18 subunit. To assess the feasibility of human gene therapy of LAD, we ***transduced*** granulocyte colony-stimulating factor (G-CSF)-mobilized, CD34+ peripheral blood stem cells derived from a patient with the severe form of LAD using supernatant from the ***retroviral*** vector PG13/LgCD18. The highest ***transduction*** frequencies (31%) were found after exposure of the cells to ***retroviral*** vector on a substrate of recombinant fibronectin fragment CH-296 in the presence of growth factors interleukin-3 (IL-3), IL-6, and stem cell factor. When the phenotype of the ***transduced*** cells was monitored by fluorescence-activated cell sorting following in vitro differentiation with growth factors G-CSF and granulocyte-macrophage CSF (GM-CSF), CD11a surface expression was detected immediately after ***transduction*** . CD11b and CD11c were expressed at low levels immediately following ***transduction*** , but increased over 3 weeks in culture. Adhesion of the ***transduced*** cells was nearly double that of nontransduced cells in a cell adhesion assay using human umbilical vein endothelial cells. ***Transduced*** cells also demonstrated the ability to undergo a respiratory burst in response to opsonized zymosan, a CD11/CD18-dependent ligand. These experiments show that ***retrovirus*** -mediated gene transfer of the CD18 subunit complements the defect in LAD CD34+ cells resulting in CD11/CD18 surface expression, and that the differentiated myelomonocytic cells derived from the ***transduced*** LAD CD34+ cells display CD11/CD18-mediated adhesion function. These results indicate that ex vivo gene transfer of CD18 into LAD CD34+ cells, followed by re-infusion of the ***transduced*** cells, may represent a therapeutic approach to LAD.

L16 ANSWER 5 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 1998:298245 BIOSIS
DN PREV199800298245
TI Improved ***adherence*** of genetically modified endothelial cells to small-diameter expanded polytetrafluoroethylene grafts in a canine model.
AU Falk, Jeffrey; Townsend, Laurace E.; Vogel, L. Michelle; Boyer, Michael; Olt, Sarah; Wease, Gary L.; Trevor, Katrina T.; Seymour, Marilyn; Glover, John L. (1); Bendick, Philip J.
CS (1) William Beaumont Hosp., 3601 W. Thirteen Mile Rd., Royal Oak, MI 48073 USA
SO Journal of Vascular Surgery, (***May, 1998***) Vol. 27, No. 5, pp. 902-909.
ISSN: 0741-5214.
DT Article
LA English
AB Purpose: A significant limitation to using genetically modified endothelial cells (ECs) to seed prosthetic grafts before implantation has been poor cell ***adherence*** to the graft lumen. Methodologic changes to improve cell ***adherence*** were evaluated in a canine carotid interposition graft model using 4 mm interior diameter expanded polytetrafluoroethylene. Methods. ECs harvested from external jugular veins were grown in culture, with 80% of the cells from each culture ***transduced*** by incubation with an LXSN-type ***retroviral*** vector carrying a gene for human prourokinase and a neomycin resistance gene for selection in antibiotic G418. Control grafts had passive luminal coating with fibronectin and were seeded with ***transduced*** ECs immediately after G418 selection; these grafts were incubated for 2 days before implantation. Experimental grafts had fibronectin forcefully squeezed through the interstices and were seeded with ECs that had recovered in culture for 5 days after G418 selection; these grafts were incubated for 4 days before implantation. For each control (n = 9) and experimental (n = 12) graft, a graft prepared in the same fashion but seeded with the remaining autologous nontransduced cells was placed in the contralateral carotid artery. Grafts were explanted after 30 days and were evaluated for patency, thrombus-free surface area, and cell-free surface area. Results: No significant differences in patency rates were seen between any groups. The thrombus-free surface area was improved for experimental grafts (90%) compared with control grafts (76%), but this improvement did not achieve statistical significance. The cell-free surface area for ***transduced*** cells on experimental grafts was 65% compared with 96% for control grafts (p = 0.021) and was comparable with that for nontransduced cells on both control grafts (62%) and experimental grafts (51%; p = 0.201). Conclusions. ***Adherence*** of genetically modified endothelial cells to small-diameter expanded polytetrafluoroethylene grafts in an in vivo physiologic flow model is significantly improved when cells have a more prolonged recovery from G418 selection, when the graft lumen is more uniformly coated with fibronectin before EC seeding, and when seeded grafts are left longer in culture before implantation to develop cell lining stability. The short-term patency rate of these seeded grafts is not affected by increased cell retention; long-term graft patency data and luminal healing require further evaluation.

L16 ANSWER 6 OF 46 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 5
AN 1998:390825 CAPLUS
DN 129:156689
TI ***Retroviral*** -mediated marker gene transfer in hematopoiesis-supportive marrow stromal cells
AU Bulabois, Claude-Eric; Yerly-Motta, Veronique; Mortensen, Borge T.; Fixe, Philippe; Remy-Martin, Jean-Paul; Herve, Patrick; Tiberghien, Pierre; Charbord, Pierre
CS Etablissement de Transfusion Sanguine de Franche-Comte, Besancon, Fr.
SO J. Hematother. (***1998***), 7(3), 225-239
CODEN: JOEMEL; ISSN: 1061-8128
PB Mary Ann Liebert, Inc.
DT Journal
LA English
AB A Moloney-derived ***retrovirus*** contg. both LacZ and NeoR genes (G1BgSVNa from Genetic Therapy, Inc.), was used to ***transduce*** human and murine bone marrow stromal cells. Different kinds of stromal cells that were able to support hematopoiesis were ***transduced*** by incubation for 24 h in the presence of virus-contg. supernatant. Semiconfluent layers of MRC-5 (human, myofibroblastic, fetal, pulmonary) and MS-5 (murine, myofibroblastic, medullary) cells were successfully ***transduced*** after one 24-h incubation, as demonstrated by G418 resistance and Escherichia coli .beta.-galactosidase staining. In contrast, human stromal cells, purified from primary confluent layers grown for 3-4 wk, could not be ***transduced*** . However, stromal cells generated after 10-12 days in culture from Stro-1 + and 1B10 + stromal precursors were successfully ***transduced*** in the presence of basic fibroblast growth factor. ***Transduced*** stromal cells maintained a myofibroblastic phenotype, although with a decreased no. of .alpha.-SM actin-pos. microfilaments in MS-5 cells. The ability to support the generation of stroma- ***adherent*** colony-forming cells from cocultured cord blood CD34 + cells after 4 wk in culture was similar before and after ***transduction*** and G418 selection. In conclusion, human primary stromal precursors can be efficiently ***transduced*** , and the stromal cell phenotype and function are not significantly altered after ***retroviral*** -mediated transfer of marker genes.

L16 ANSWER 7 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 1998:511664 BIOSIS
DN PREV199800511664
TI Selection and extended growth of murine epidermal stem cells in culture.
AU Bickenbach, Jackie R. (1); Chism, Emily
CS (1) Dep. Anat. and Cell Biol., Coll. Med., 51 Newton Road, Univ. Iowa, Iowa City, IA 52242-1109 USA
SO Experimental Cell Research, (***Oct. 10, 1998***) Vol. 244, No. 1, pp. 184-195.
ISSN: 0014-4827.
DT Article
LA English
AB Continuously renewing epithelia contain small undifferentiated stem cells capable of self-renewal and maintenance of the differentiating cell population. In murine epidermis stem cells have been identified as label-retaining cells (LRCs) by long-term retention of tritiated thymidine or BrdU. It has been suggested that epidermal stem cells ***adhere*** to basement membranes through differential expression of specific integrins. To determine whether we could use a specific integrin to enrich for murine epidermal stem cells, we tested ***adherence*** of LRCs to several substrates. Regardless of the substrate used, approximately 10% of total basal cells and 100% of LRCs ***adhered*** in 10 min. In our medium specifically formulated for murine keratinocytes, rapidly ***adherent*** stem cells formed large colonies and could be used to form a structurally complete epidermis in organotypic culture. They showed a fivefold greater transient transfection efficiency than total basal cells, and when individual ***adherent*** cells were ***transduced*** with a ***retroviral*** vector, they formed large clones. Although these stem cells grew more slowly than the total basal cell population, they could be subcultured more times. Our results indicate that murine epidermal stem cells can be selected by rapid attachment to a substrate, but not by one specific integrin, and that they can be expanded in culture if the appropriate conditions are maintained.

L16 ANSWER 8 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 1998:448065 BIOSIS
DN PREV199800448065
TI Feasibility of double-expression retrovival vector using complement regulatory factor gene.
AU Hayashi, Shuji (1); Emi, Nobuhiko; Okada, Hidechika; Yokoyama, Itsuo; Takagi, Hiroshi
CS (1) Dep. Surg. II, Nagoya Univ. Sch. Med., 65 Tsurumai-cho, Showa-ku, Nagoya 466 Japan
SO Journal of Surgical Research, (***July 15, 1998***) Vol. 78, No. 1, pp. 64-67.
ISSN: 0022-4804.
DT Article
LA English
AB The donor source of vascular endothelial cells for hybrid blood vessels seeded with genetically engineered endothelial cells is generally considered to be autologous. The purpose of this study was to determine whether porcine endothelial cells ***transduced*** with double-expression ***retroviral*** vector using complement-resistant gene could be substituted for autologous endothelial cells. Decay-accelerating factor (DAF) and tissue plasminogen activator (tPA) cDNA were inserted into ***retroviral*** vector with homologous restriction factor 20 cDNA as a complement regulatory factor gene. Porcine aortic endothelial cells were ***transduced*** with these double-expression ***retroviral*** vectors, followed by the complement-dependent selection. Porcine endothelial cells ***transduced*** with double-expression ***retroviral*** vectors showed a high gene expression of both DAF and tPA. Complement-dependent cytotoxicity and ***adherence*** of U937 were significantly inhibited by the ***transduction*** of double-expression vectors with complement regulatory factor gene. Double-expression ***retroviral*** vector using complement regulatory factor gene was efficacious in substituting porcine endothelial cells for the autologous endothelial cells.

L16 ANSWER 9 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8
AN 1998:26590 BIOSIS
DN PREV199800026590
TI Gene transfer into marrow repopulating cells: Comparison between amphotropic and gibbon ape leukemia virus pseudotyped ***retroviral*** vectors in a competitive repopulation assay in baboons.
AU Kiem, Hans-Peter (1); Heyward, Scott; Winkler, Aaron; Potter, Jennifer; Allen, James M.; Miller, A. Dusty; Andrews, Robert G.
CS (1) Fred Hutchinson Cancer Res. Cent., 1100 Fairview Ave. N, Seattle, WA 98109-1024 USA
SO Blood, (***Dec. 1, 1997***) Vol. 90, No. 11, pp. 4638-4645.
ISSN: 0006-4971.
DT Article
LA English
AB Many diseases might be treated by gene therapy targeted to the hematopoietic system, but low rates of gene transfer achieved in humans

and large animals have limited the application of this technique. We have developed a competitive hematopoietic repopulation assay in baboons to evaluate methods for improving gene transfer and have used this method to compare gene transfer rates for ***retroviral*** vectors having an envelope protein (pseudotype) from amphotropic murine ***retrovirus*** with similar vectors having an envelope protein derived from gibbon ape leukemia virus (GALV). We hypothesized that vectors with a GALV pseudotype might perform better based on our previous work with cultured human hematopoietic cells. CD34+ marrow cells from each of four untreated baboons were divided into two equal portions that were cocultivated for 48 hours with packaging cells producing equivalent titers of either amphotropic or GALV pseudotyped vectors containing the neo gene. The vectors contained small sequence differences to allow differentiation of cells genetically marked by the different vectors. Nonadherent and ***adherent*** cells from the cultures were infused into animals after they received a myeloablative dose of total body irradiation. Polymerase chain reaction (PCR) analysis for neo gene-specific sequences in colony-forming unit-granulocyte-macrophage from cell populations used for transplant showed gene transfer rates of 2.7%, 7.1%, >15%, and 3.9% with the amphotropic vectors and 7.1%, 11.3%, >15%, and 26.4% with the GALV pseudotyped vector. PCR analysis of peripheral blood and marrow cells after engraftment showed the neo gene to be present in all four animals analyzed at levels between 0.1% and 5%. Overall gone transfer efficiency was higher with the GALV-pseudotyped vector than with the amphotropic vectors. Southern blot analysis in one animal confirmed a gene transfer efficiency of between 1% and 5%. The higher gene transfer efficiency with the GALV-pseudotyped vector correlated With higher levels of GALV receptor RNA compared with the amphotropic receptor in CD34+ hematopoietic cells. These results show that GALV-pseudotyped vectors are capable of ***transducing*** baboon marrow repopulating cells and may allow more efficient gene transfer rates for human gene therapy directed at hematopoietic cells. In addition, our data show considerable differences in gene transfer efficiency between individual baboons, suggesting that a competitive repopulation assay will be critical for evaluation of methods designed to improve gene transfer into hematopoietic stem cells.

L16 ANSWER 10 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
9
AN 1998:44050 BIOSIS
DN PREV199800044050
TI Fibroblast growth factor-2 inhibits endothelial cell apoptosis by Bcl-2-dependent and independent mechanisms.
AU Karsan, Aly (1); Yee, Esther; Poirier, Guy G.; Zhou, Ping; Craig, Ruth; Harlan, John M.
CS (1) McDonald Research Lab., Room 292, St. Paul's Hosp., 1081 Burrard St., Vancouver, BC V6Z 1Y6 Canada
SO American Journal of Pathology, (***Dec., 1997***) Vol. 151, No. 6, pp. 1775-1784.
ISSN: 0002-9440.
DT Article
LA English
AB Intact endothelium acts as a sensor and ***transducer*** of signals and also provides a nonthrombogenic surface at the blood-vascular wall interface. Hence, mechanisms that maintain the integrity of the endothelium are of interest in physiological and pathological states. In this study we show that apoptosis induced by growth factor and serum deprivation of endothelial cells occurs at all phases of the cell cycle and can be blocked by fibroblast growth factor-2 (FGF-2) independently of its mitogenic activity. As the Bcl-2 family of proteins plays a prominent role in regulating cell survival, we attempted to identify Bcl-2 homologues expressed in endothelial cells. Here we demonstrate that, in addition to the previously identified A1, four other members of the Bcl-2 family, Bcl-2, Mcl-1, Bcl-XL, and Bax, are expressed in endothelial cells. Of these family members, only Bcl-2 is induced by FGF-2. Overexpression of Bcl-2, using a ***retroviral*** vector, protects endothelial cells from serum and growth factor deprivation. There is no difference in FGF-2-induced proliferation between Bcl-2-overexpressing cells and those ***transduced*** with the empty ***retroviral*** vector. At early time points Bcl-2 is not up-regulated, but FGF-2 still has a protective effect. However, FGF-2 protects only ***adherent*** endothelial cells but not those that are cultured in suspension. The early effect of FGF-2 is dependent on tyrosine phosphorylation but not on activation of the MAP kinase pathway. Thus, FGF-2 inhibits endothelial cell apoptosis by Bcl-2-dependent and independent mechanisms.

L16 ANSWER 11 OF 48 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 10
AN 1997:555032 CAPLUS
DN 127:243768
TI LacZ and interleukin-3 expression in vivo after ***retroviral*** ***transduction*** of marrow-derived human osteogenic mesenchymal progenitors
AU Allay, James A.; Dennis, James E.; Haynesworth, Stephen E.; Majumdar, Manas K.; Clapp, D. Wade; Shultz, Leonard D.; Caplan, Arnold I.; Gerson, Stanton L.
CS Departments of Medicine, Biology, The Ireland Cancer Center, Ire.
SO Hum. Gene Ther. (***1997***), 8(12), 1417-1427
CODEN: HGTHE3; ISSN: 1043-0342
PB Liebert
DT Journal
LA English
AB Human marrow-derived mesenchymal progenitor cells (hMPCs), which have the capacity for osteogenic and marrow stromal differentiation, were ***transduced*** with the myeloproliferative sarcoma virus (MPSV)-based ***retrovirus*** , vM5LacZ, that contains the LacZ and neo genes. Stable ***transduction*** and gene expression occurred in 18% of cells. After culture expansion and selection in G418, approx. 70% of neor hMPCs co-expressed LacZ. G418-selected hMPC retain their osteogenic potential and form bone in vivo when seeded into porous calcium phosphate ceramic cubes implanted s.c. into SCID mice. LacZ expression was evident within osteoblasts and osteocytes in bone developing within the ceramics 6 and 9 wk after implantation. Likewise, hMPCs ***transduced*** with human interleukin-3 (hIL-3) cDNA, ***adhered*** to ceramic cubes and implanted into SCID mice, formed bone and secreted detectable levels of hIL-3 into the systemic circulation for at least 12 wk. These data indicate that genetically ***transduced*** , culture-expanded bone marrow-derived hMPCs retain a precursor phenotype and maintain similar levels of transgene expression during osteogenic lineage commitment and differentiation in vivo. Because MPCs have been shown to differentiate into bone, cartilage, and tendon, these cells may be a useful target for gene therapy.

L16 ANSWER 12 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
11
AN 1997:511593 BIOSIS
DN PREV199799810798
TI Effect of ***retroviral*** ***transduction*** on human endothelial cell phenotype and adhesion to Dacron vascular grafts.
AU Jankowski, Ronald J.; Severyn, Donald A.; Vorp, David A.; Wagner, William R. (1)
CS (1) Dep. Surgery, C-813 PUH, Univ. Pittsburgh Med. Cent., 200 Lothrop Street, Pittsburgh, PA 15213 USA
SO Journal of Vascular Surgery, (1997) Vol. 26, No. 4, pp. 676-684.
ISSN: 0741-5214.
DT Article
LA English
AB Purpose: ***Retroviral*** ***transduction*** for genetic enhancement of endothelial cell (EC) antithrombotic phenotype offers potential for improving the clinical success of vascular graft seeding; however, application of this technique may bring concomitant alteration in cell functionality. Methods: Human microvascular ECs were ***transduced*** with a ***retroviral*** vector encoding for the marker gene beta-galactosidase. ***Transduced*** endothelial cells (rtECs) an nontransduced endothelial cells (ntECs) were evaluated by flow cytometry for expression of intercellular adhesion molecule (ICAM)-1 and tissue factor (TF) on both smooth (coverslips) and graft (Dacron, 6 mm inside diameter) surfaces under static and shear exposed conditions. Graft EC retention was measured after 6-hour pulsatile perfusions. Platelet and neutrophil ***adherence*** was measured on perfused coverslips. Results: Lower levels of ICAM-1 were expressed by rtECs on coverslips under both static (p lt 0.01 vs static ntECs) and shear exposed conditions (p lt 0.01 vs static and shear ntECs). Accordingly, fewer polymorphonuclear leukocytes ***adhered*** to rtEC monolayers (p lt 0.01 vs ntECs). No difference in ICAM-1 and TF expression by static graft seeded rtECs and ntECs was observed. However, graft-seeded rtECs that were exposed to wall shear stress displayed less TF than sheared ntECs (p lt 0.05). ***Transduction*** did not affect EC retention to the sheared graft surface. Conclusions: These data suggest that ***retroviral*** ***transduction*** does not elicit a prothrombotic/proinflammatory phenotype, rather indices of these states appear in some conditions to be reduced. Further, ***transduction*** does not adversely affect EC ***adherence*** to Dacron graft surfaces under arterial hemodynamics.

L16 ANSWER 13 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
12
AN 1997:297856 BIOSIS
DN PREV199799597059
TI In vitro maintenance and ***retroviral*** ***transduction*** of human myeloma cells in long-term marrow cultures.
AU Stewart, A. Keith (1); Prince, H. Miles; Cappe, Darrin; Chu, Peter; Lutzko, Carolyn; Sutherland, D. Robert; Dube, Ian D.
CS (1) mlw 2-025, Toronto Hosp., Gen. Div., 657 University Ave., Toronto, ON M5G 2C4 Canada
SO Cancer Gene Therapy, (1997) Vol. 4, No. 3, pp. 148-156.
ISSN: 0929-1903.
DT Article
LA English
AB One objective of clinical gene marking trials in multiple myeloma (MM) is to determine the extent to which relapse after stem cell transplant is attributable to contamination of the autograft with myeloma cells. A requirement in these studies is ex vivo genetic marking of malignant cells present in autografts which are derived from patients exposed to significant prior chemotherapy. We evaluated gene marking of clonogenic myeloma cells in marrow aspirates from 14 patients with MM. To effect gene transfer we utilized a long-term marrow culture (LTMC) system previously shown to facilitate gene transfer into a spectrum of hematopoietic progenitor and stem cells. ***Transduction*** of cells in LTMC was performed by multiple supernatant exposure. At LTMC initiation and after 21 days of culture malignant cells were assessed by morphology, flow cytometry, and polymerase chain reaction (PCR). The mean number of day 21 LTMC ***adherent*** layer-derived granulocyte/macrophage progenitors as a percentage of the original inoculum was within the normal range for this technique. The efficiency of ***transduction*** of normal hematopoietic progenitors as determined by the number of colonies positive for proviral DNA by PCR, G418 resistance, and X-gal staining was also within the expected range: 65%, 44% and 23%, respectively. Thus, there was no evidence that prior chemotherapy exposure or malignant cell contamination compromised cell survival or gene transfer efficiency in LTMC. All patients retained plasma cells in LTMCs for the duration of the 21-day culture period. Molecular analysis confirmed the persistence of clonal IgVH gene rearrangements in day 21 LTMC-derived DNA from 8 of 12 informative patients (50%). PCR using allele-specific primers when available confirmed the specificity of IgVH rearrangements for the myeloma clone. In 2 of the 14 patients, expansion of clonogenic cells was demonstrated in LTMC. In both cases there was strong evidence for transfer of reporter genes (neo' and LacZ) into the myeloma clone: morphologically abnormal G418-resistant colonies demonstrated intense staining for beta-galactosidase, and cytospin preparations showed 100% plasma cells with monoclonal heavy and light chain restriction. In one patient, individual colonies positive for beta-galactosidase bore a cytogenetic abnormality characteristic of the patient's myeloma clone. PCR of DNA from pooled plasma cell colonies using tumor-specific CDR3 primers was positive. Our results demonstrate the maintenance of myeloma cells in vitro for up to 21 days in LTMC. They further illustrate that these cells can be genetically marked using ***transduction*** protocols currently being tested in clinical trials of hematopoietic cell gene transfer.

L16 ANSWER 14 OF 48 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1997:291244 BIOSIS
DN PREV199799590447
TI Dysregulated Mpl-ligand production by hemopoietic cells induces a fatal myeloproliferative syndrome in mice.
AU Villeval, J.-L. (1); Cohen-Solal, K.; Tulliez, M.; Giraudier, S.; Guichard, J.; Burstein, S.; Cramer, E. M.; Vainchenker, W.; Wendling, F.
CS (1) Dana Farber Cancer Inst., Room D936, 44 Binney St., Boston 02115, MA USA
SO Hematology and Cell Therapy, (1997) Vol. 39, No. 2, pp. 117-118.
ISSN: 1269-3286.
DT Article
LA English
SL English; French
AB To evaluate the effects of long-term high-dose exposure to Mpl-ligand also

called thrombopoietin (TPO), C57BL/6J murine marrow cells were infected with a ***retrovirus*** carrying the murine TPO gene. Mice were treated 4 days by 5-FU and marrow cells were then infected by coculture using a MPZen vector containing the murine TPO cDNA. Non ***adherent*** marrow cells were transplanted into lethally irradiated recipients. A majority of hematopoietic cells in the marrow, spleen, thymus and blood was ***transduced*** by the ***retroviral*** vector, one and three months after reconstitution. Plasma TPO activity in transplanted mice was extremely high (104 U/ml). A disease with two distinct steps was observed. During the two first months after transplantation, platelet (plt) and white blood cell (WBC) counts increased 4- and 10-fold, respectively. Abnormal platelet size and granules were observed. Spleen weight increased 4-fold and marrow cellularity decreased 5-fold. Histology revealed hyperplasia of the megakaryocytic and myeloid cells. Total numbers of CFU-MK and CFU-GM increased. In contrast, the hematocrit progressively fell accompanied by a decrease in the erythroblasts and CFU-E numbers. Beginning two months after transplantation, plt and WBC numbers also declined. Thrombocytopenia was noted 5 months after transplantation. The Hcts continued to decrease. Few cells were isolated from the marrow cavities and spleens. Histology revealed fibrosis of the marrow and spleen and significant osteosderosis of the marrow. An extramedullary hematopoiesis was observed in numerous organs such as the liver or the kidney. Total numbers of progenitors were very low in hematopoietic organs. Mice died 7 months after transplantation with severe pancytopenia. Two early deaths were observed with a marked increase in blast cells. This disorder was transplantable into secondary recipients who developed an attenuated form of the disease similar to the one previously described (Yan et al (1995) Blood 86: 4025). In conclusion, dysregulated TPO production by hemopoietic cells in mice results in a fatal myeloproliferative disease which mimics the clinical evolution of idiopathic myelofibrosis observed in man.

L16 ANSWER 15 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
13
AN 1997:362291 BIOSIS
DN PREV199799654224
TI Effect of rhBMP-2 on the osteogenic potential of bone marrow stromal cells from an osteogenesis imperfecta mouse (oim.
AU Balk, M. L.; Bray, J.; Day, C.; Epperly, M.; Greenberger, J.; Evans, C. H.; Niyibizi, C. (1)
CS (1) Musculoskeletal Res. Cent., Dep. Orthop. Surg., 986 Scaife Hall, Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261 USA
SO Bone (New York), (1997) Vol. 21, No. 1, pp. 7-15.
ISSN: 8756-3282.
DT Article
LA English
AB To understand whether osteogenesis imperfecta (OI) could result from defective differentiation of osteoprogenitor cells, we investigated the osteogenic potential of bone marrow stromal cells from a mouse model of human OI (oim). Bone marrow was flushed from the femurs and tibias of oim and normal littermates using a syringe with Dulbecco's modified Eagle's medium, and cells were allowed to ***adhere*** to flasks. ***Adherent*** cells were trypsinized and passaged weekly at a 1:4 split. The established stromal cells were assessed for collagen synthesis, alkaline phosphatase, and osteocalcin production in the presence or absence of rhBMP-2. The stromal cells were also assessed for mineralization by Von-Kossa staining and for exogenous gene transfer using adeno-lacZ and a ***retroviral*** vector. The bone marrow stromal cells from oim mice synthesized alpha-1(I) homotrimers as expected, whereas the stromal cells from the normal littermates synthesized alpha-1(I)-2-alpha-2(I) heterotrimers. The bone marrow stromal cells exhibited low levels of alkaline phosphatase activity under basal conditions; upon treatment with rhBMP-2, the level of the alkaline phosphatase activity increased approximately 40-fold. Cytochemical staining of the cells confirmed the expression of alkaline phosphatase by the oim stromal cells and its augmentation by rhBMP-2. Osteocalcin production in the stromal cells was also enhanced approximately threefold by rhBMP-2. oim stromal cells grown in the presence of beta-glycerophosphate and ascorbic acid demonstrated Von-Kossa-positive solid deposits after 3 weeks in culture. Ten days after infection with adeno-lacZ, approximately 70% of oim stromal cells expressed the transgene product, and after infection with a ***retrovirus*** , approximately 20% of the cells expressed the transgene. These data indicate that bone marrow stromal cells have osteogenic potential, and also the potential to be ***transduced*** with exogenous genes. Under basal conditions, however, the stromal cells from oim mice exhibited significantly lower levels of alkaline phosphatase activity than their normal littermates.

L16 ANSWER 16 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
14
AN 1996:412619 BIOSIS
DN PREV199699134975
TI Functional re-expression of laminin-5 in laminin-gamma-2-deficient human keratinocytes modifies cell morphology, motility, and adhesion.
AU Gagnoux-Palacios, Laurent; Vailly, Joelle; Durand-Clement, Monique; Wagner, Ernst; Ortonne, Jean-Paul; Meneguzzi, Guerrino (1)
CS (1) INSERM U385, U.F.R. Med., Av Valombrose, 06107 Nice cedex 2 France
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 31, pp. 18437-18444.
ISSN: 0021-9258.
DT Article
LA English
AB Herlitz junctional epidermolysis bullosa (H-JEB) is characterized by a reduced ***adherence*** of keratinocytes consequent to deficient expression of the extracellular adhesive ligand laminin-5. To complement the genetic defect causing H-JEB, we transferred an eukaryotic cassette expressing the cDNA for the gamma-2 chain of laminin-5 into H-JEB keratinocytes in which the expression of the polypeptide is hampered by a homozygous mutation generating a premature termination codon. Transfection using adenovirus-polylysin-transferrin-DNA complexes resulted in a transient synthesis of the recombinant laminin gamma-2 chain that associated with the endogenous alpha-3 and beta-3 chains to form laminin-5 molecules readily deposited on the tissue culture substrate. Furthermore, ***retroviral*** -mediated ***transduction*** of the gamma-2 cDNA yielded persistent expression and polarized secretion of laminin-5. The protein incorporated into the basement membrane produced by the revertant cells inoculated subcutaneously in nude mice. In these transfectants, re-expression of laminin-5 induced changes in cell morphology and reorganization of focal adhesions that assumed the shape and distribution of the counterparts detected in normal keratinocytes. These observations correlated with an enhanced cell-substrate adhesion and a reduced motility of the transfected cells. Our results demonstrate that a restored expression of laminin-5 induces a phenotypic reversion of genetically altered H-JEB keratinocytes and open new perspectives to the analysis of the mechanisms regulating adhesion of epithelial cells.

L16 ANSWER 17 OF 46 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 15
AN 1997:28572 CAPLUS
DN 126:70098
TI Two-step gene transfer using an adenoviral vector carrying the CD4 gene and human immunodeficiency viral vectors
AU Miyake, Koichi; Tohyama, Takashi; Shimada, Takashi
CS Department of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, 113, Japan
SO Hum. Gene Ther. (***1996***), 7(18), 2281-2288
CODEN: HGTHE3; ISSN: 1043-0342
PB Liebert
DT Journal
LA English
AB Human immunodeficiency virus-1 (HIV-1) belongs to the lentivirus subfamily of ***retroviruses*** and has several interesting features, including T cell tropism and the ability to infect nondividing cells. Replication-incompetent HIV vectors were developed and were shown to be capable of targeted gene transfer into CD4+ T cells. This strict T cell tropism may be important for the development of gene therapy of acquired immunodeficiency syndrome (AIDS), but it hampers the use of the HIV vector for other gene transfer applications. To expand the host range of the HIV vector, we established the two-step gene transfer system, which allows us to ***transduce*** non-T cells stably. In the first step, the CD4 gene was introduced into target cells using a replication-defective adenoviral vector. Transient but high-level expression of CD4 mols. was detected in both ***adherent*** and floating cells. In the subsequent step, the cells were incubated with HIV vectors. Stable integration of the HIV vector was demonstrated in cells ***transduced*** with the adenoviral vector. These results indicate that transient expression of CD4 mols. by the adenoviral vector is sufficient to render non-T cells susceptible to HIV-mediated gene transfer. This two-step gene transfer strategy may be used as a general method to ***transduce*** various types of human cells stably including non-dividing cells.

L16 ANSWER 18 OF 46 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 16
AN 1996:742787 CAPLUS
DN 126:43283
TI Preclinical assessment of human hematopoietic progenitor cell ***transduction*** in long-term marrow cultures
AU Dube, Ian D.; Kruth, Steven; Abrams-Ogg, Anthony; Kamel-Reid, Suzanne; Lutzko, Carolyn; Nanji, Shaherose; Ruedy, Christine; Singaraja, Roshni; Wild, Anthony; et al.
CS Sunnybrook Health Science Centre, University Toronto, Toronto, ON, M4N 3M5, Can.
SO Hum. Gene Ther. (***1996***), 7(17), 2089-2100
CODEN: HGTHE3; ISSN: 1043-0342
PB Liebert
DT Journal
LA English
AB Long-term marrow cultures (LTMCs) were established from 27 human marrows. Hematopoietic cells were subjected to multiple rounds of exposure to ***retroviral*** vectors during 3 wk of culture. Seven different ***retroviral*** vectors were evaluated. LTMCs were assessed for viability, replication-competent ***retrovirus*** , progenitors capable of proliferating in immune-deficient mice, and gene transfer. The av. no. of ***adherent*** cells and committed granulocyte-macrophage progenitors (CFU-GM) recovered from LTMCs was 28% and 11% of the input totals, resp. There was no evidence by marker rescue assay or polymerase chain reaction (PCR) of replication-competent virus prodn. during LTMC. No toxicity to cellular proliferation due to the ***transduction*** procedure was obsd. The ***adherent*** layers of LTMCs exposed to ***retroviral*** vectors were pos. for proviral DNA by PCR and by Southern blot anal. Fifty-three percent of 1,427 individual CFU-GM from ***transduced*** LTMC ***adherent*** layers were pos. for vector-derived DNA. For neor-contg. vectors, the av. G418 resistance was 28% of 1,393 LTMC-derived CFU-GM. Forty percent of 187 tissues from 30 immune-deficient mice injected with human LTMC cells were pos. for human DNA 4-5 wk after adoptive transfer. These studies indicate that multiple exposures of human LTMCs to ***retroviral*** vectors result in consistent and reproducible LTMC viability and gene transfer into committed progenitors. These results further support the use of ***transduced*** LTMC cells in clin. trials of hematopoietic stem cell gene transfer.

L16 ANSWER 19 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
17
AN 1996:187639 BIOSIS
DN PREV199698743768
TI Seeding of vascular grafts with genetically modified endothelial cells: Secretion of recombinant TPA results in decreased seeded cell retention in vitro and in vivo.
AU Dunn, Peter F.; Newman, Kurt D.; Jones, Michael; Yamada, Izumi; Shayani, Vafa; Virmani, Renu; Dichek, David A. (1)
CS (1) Gladstone Inst. Cardiovasc. Dis., PO Box 419100, San Francisco, CA 94141-9100 USA
SO Circulation, (1996) Vol. 93, No. 7, pp. 1439-1446.
ISSN: 0009-7322.
DT Article
LA English
AB Background: Seeding of small-diameter vascular grafts with endothelial cells (ECs) genetically engineered to secrete fibrinolytic or antithrombotic proteins offers the potential to improve graft patency rates. Methods and Results: Sheep venous ECs were ***transduced*** with a ***retroviral*** vector encoding human tissue plasminogen activator (TPA). The ECs were seeded onto 4-mm-ID synthetic (Dacron) grafts. Retention of the seeded ECs was measured 2 hours after placement of the seeded grafts both in vitro in a nonpulsatile flow system and in vivo (in sheep) as femoral and carotid interposition grafts. On exposure to flow in vitro, ECs ***transduced*** with TPA were retained at a significantly lower rate (median, 67%) than either untransduced ECs (81%) or ECs ***transduced*** with a control ***retroviral*** vector producing beta-galactosidase (beta-Gal) (80%) (P lt .05 for TPA versus either control). On implantation in vivo, ECs ***transduced*** with TPA were retained at a very low rate (median, 0%), significantly less than the retention of ECs ***transduced*** with the beta-Gal vector (32%; P lt .00001). Decreased in vivo retention of ECs ***transduced*** with

TPA correlated modestly with increased in vitro cellular passage level (r-2=.48; *P lt .0001*) but not with in vivo blood flow rate (P=.45). Addition of the protease inhibitor aprotinin to the cell culture and graft perfusion media resulted in a significant (*P lt .05*) *increase in in vitro retention of ECs* ***transduced*** with TPA. Conclusions: Increased TPA expression significantly decreases seeded EC ***adherence*** in vitro and in vivo. Gene therapy *strategies for decreasing* graft thrombosis may require expression of antithrombotic molecules that lack proteolytic activity.

L16 ANSWER 20 OF 46 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 18
AN 1996:459837 CAPLUS
DN 125:131529
TI Frequency analysis of multidrug resistance-1 gene transfer into *human primitive hematopoietic* progenitor cells using the cobblestone area-forming cell assay and detection of vector-mediated P-glycoprotein expression by rhodamine-123
AU *Fruehauf, S.*; Breems, D.A.; Knaan-Shanzer, S.; Brouwer, K.B.; Haas, R.; Lowenberg, B.; Nooter, K.; Ploemacher, R.E.; Valerio, D.; Boesen, J.J.B.
CS Department of *Medical Biochemistry, University* of Leiden, Rijswijk, 2280 GG, Neth.
SO Hum. Gene Ther. (***1996***), 7(10), 1219-1231
CODEN: HGTHE3; ISSN: 1043-0342
DT Journal
LA English
AB Transfer of the multidrug resistance-1 (MDR1) gene into hematopoietic progenitor cells may reduce myelotoxicity of MDR1-related cytotoxic *agents and therefore allow dose intensification*. Mobilized peripheral blood progenitor cells (PBPC) can be obtained in ample quantity and are a suitable target cell population. *CD34-selected PBPC samples (n = 6) were* ***transduced*** with cell-free supernatant (SNT) of a cell line producing recombinant ***retrovirus*** contg. the human MDR1 gene. *Limiting-diln. long-term cultures were employed* that allow continuous monitoring of stroma- ***adherent*** cobblestone areas (CA) and comparison of their frequency in a 5-log range over time. MDR1 *provirus integration in CA-contg.* wells followed single-hit kinetics. According to Poisson statistics, proviral DNA was contained in 22% of unselected cobblestone area-forming *cells (CAFC) at week 6, which represent* primitive hematopoietic precursors. In comparison, 1.0 .+-. 0.44% (mean .+-. SEM) of week-6 CAFC were expressing P-glycoprotein at sufficient levels to convey *vincristine resistance*, suggesting low expression of the ***retroviral*** vector or splicing of the vector-derived mRNA in hematopoietic progenitor cells. Next we analyzed *lineage-committed progenitors*. The proviral DNA was detectable in 20-66% of colony-forming units granulocyte-macrophage (CFU-GM) while corresponding percentages (25-52%) of *CD34+ PBPC were in the S/G2M phase* of the cell cycle at the end of the ***transduction*** period. The proportion of vincristine-resistant CFU-GM was similar to the *CAFC data and no significant differences* were found between various MDR1-SNT ***transduction*** schedules whereas MDR1 co-cultivation, which served as a pos. control, yielded significantly *higher proportions of* resistant colonies (5.3 .+-. 1.4%, IL-3, 96 h, p .ltoreq. 0.05). Assessment of rhodamine-123 (Rh-123) efflux in the myelo-monocytic progeny of *MDR1-* ***transduced*** *cells mirrored* the colony assay results in the SNT and co-cultivation groups. Less culture effort was required in the Rh-123 assay and functional characterization of the transferred *P-glycoprotein* was possible using cyclosporin A. Further development toward an effective MDR1 gene therapy should be facilitated by the CAFC assay, which allows estn. of the ***retroviral*** *gene transfer* frequency into primitive hematopoietic cells, and by the Rh-123 assay, which permits tractable side-by-side assessments of numerous MDR1 ***transduction*** *protocols or different MDR1-SNT* lots.

L16 ANSWER 21 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
19
AN 1996:437743 BIOSIS
DN PREV199699151349
TI Colocalization of ***retrovirus*** and *target cells on specific* fibronectin fragments increases genetic ***transduction*** of mammalian cells.
AU Haenenberg, Helmut; *Xiao, Xiang Li*; Dilloo, Dagmar; Hashino, Kimikazu; Kato, Ikunoshin; Williams, David A. (1)
CS (1) Sect. Pediatric Hematol./Oncol., Herman B. No. *Wells Cent. Pediatric* Res., *Riley Hosp.* Children, Indiana Univ. Sch. Med., 702 Barnhill Drive, Indianapolis, IN 46202-5225 USA
SO Nature Medicine, (1996) *Vol. 2, No. 8, pp. 876-882.*
ISSN: 1078-8956.
DT Article
LA *English*
AB Hematopoietic cells are important targets for genetic modification with ***retroviral*** vectors. Attempts at human *gene therapy of stem* cells have achieved limited success partly because of low gene transfer efficiency. Chymotryptic fragments of the extracellular matrix molecule fibronectin *used during infection have been* shown to increase ***transduction*** of human hematopoietic progenitor cells. Here, we demonstrate that this enhanced gene transfer into *mammalian target cells* is due to direct binding of ***retroviral*** particles to sequences within the fibronectin molecule. ***Transduction*** of mammalian cells, including murine *long-term repopulating hematopoietic* cells, is greatly enhanced when cells are ***adherent*** to chimeric fragments containing these ***retroviral*** binding sequences. In addition, *colocalization of* ***retrovirus*** and target cells on fibronectin peptides allows targeted ***transduction*** of specific cell types by exploiting unique ligand/receptor *interactions*.

L16 ANSWER 22 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
20
AN 1996:407266 BIOSIS
DN PREV199699129622
TI Fibronectin improves ***transduction*** of reconstituting *hematopoietic stem cells by* ***retroviral*** vectors: Evidence of direct viral binding to chymotryptic carboxy-terminal fragments.
AU Moritz, Thomas; Dutt, Parmesh; Xiao, Xiangli; *Carstanjen, Dirk*; Vik, *Terry*; Hanenberg, Helmut; Williams, David A. (1)
CS (1) Howard Hughes Med. Inst., Herman B. Wells Cent. Pediatric Res., Indiana *Univ. Sch. Med., 702 Barnhill Dr., Room* 2600, Indianapolis, IN 46202-5225 USA
SO Blood, (1996) Vol. 88, No. 3, pp. 855-862.
ISSN: 0006-4971.
DT Article
LA English
AB Efficient ***transduction*** of reconstituting *hematopoietic stem cells (HSC)* is currently only possible by cocultivation of target cells directly on producer cell lines, a method not applicable to human gene therapy protocols. Our *laboratory has previously shown* adhesion of primitive hematopoietic stem and progenitor cells to the carboxy-terminal 30/35-kD fragment of the extracellular matrix molecule fibronectin *(FN 30/35) (Nature 352:438, 1991)* and increased ***transduction*** of human hematopoietic progenitor cells via ***retroviral*** vectors while ***adherent*** to this fragment *(J Clin Invest 93:1451, 1994). Here we report that* (1) ***transduction*** of reconstituting murine HSC assayed 12 months after infection with ***retrovirus*** supernatant on FN 30/35 *is as effective as* cocultivation directly on producer cells; (2) recombinant ***retrovirus*** particles directly ***adhere*** to FN 30/35 in a quantitative and *dose-dependent fashion; and (3) increased* ***transduction*** efficiency on FN 30/35 does not appear to be associated with increased cell proliferation or activation of protein phosphorylation *typically induced by integrin*-fibronectin interactions. Therefore, we speculate that supernatant infection of HSC on FN 30/35 leads to colocalization of ***retrovirus*** particles and *target cells on FN 30/35* molecule with a large increase in local virus titer presented to the cell. These findings have direct and important implications for the modification of *current human gene therapy protocols*.

L16 ANSWER 23 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
21
AN 1996:472108 BIOSIS
DN PREV199699201684
TI Stromal cells maintain the radioprotective capacity of CFU-S during ***retroviral*** infection.
AU Goncalves, F.; Durbart, A.; Lacout, C.; Vainchenker, W.; Dumenil, D. (1)
CS (1) U362 INSERM, Inst. Gustave Roussy, Rue Camille Desmoulins, *94800 Villejuif France*
SO Gene Therapy, (1996) Vol. 3, No. 9, pp. 761-768.
ISSN: 0969-7128.
DT Article
LA English
AB ***Retroviral*** *vectors provide an efficient* means to introduce genes into hematopoietic stem cells. In order to develop ***retroviral*** infection protocols which preserve the *radioprotective capacity of CFU-S, we designed a clonal* hematopoietic reconstitution assay. In this assay, single CFU-S-derived colonies from bone marrow cells of 5-FU-treated mice were tested for their capacity to *prevent radiation-induced* mortality. Three parameters which may modify stem cell potential were tested in infection protocols using a ***retroviral*** vector containing the gene for *neomycin resistance: (1)* the partition of stem cells between the ***adherent*** and nonadherent fraction; (2) the replacement of the packaging cell line by a *'competent' stromal cell line; and (3) the effects of* G418 selection. All CFU-S having radioprotective capacity were found in the ***adherent*** fraction when the packaging cell line or the stromal cell line *(MS-5) chosen for its capacity* to maintain long-term bone marrow culture were used during the co-culture. The neo resistance gene was ***transduced*** into CFU-S with the same efficiency *using co-culture with the packaging* cell line or co-culture with the MS-5 cell line plus viral supernatant. However, in the presence of MS-5, a much higher proportion of *CFU-S (70% versus 30%) had radioprotective* properties, suggesting an important role for the stromal cells in the maintenance of hematopoietic reconstituting ability. Finally, G418 selection, *even for a limited period (24 h)*, significantly decreased the radioprotective capacities of CFU-S (56% versus 18%). Subsequently, hematopoietic reconstitution by single *CFU-S was quantified in a recipient mice*. The progeny of CFU-S were found at a significant level in the blood, spleen and bone marrow in 38% and 15% of mice, 1 and 3 months after transplantation, respectively. *These results demonstrate* that we have substantially improved the infection protocol. Under these conditions of infection, it is possible to conserve CFU-S properties and to ***transduce*** a gene into a stem cell with short-term hematopoietic reconstitution potential.

L16 ANSWER 24 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
22
AN 1996:320215 BIOSIS
DN PREV199699042571
TI Correction of Fanconi anemia type C phenotypic abnormalities using a clinically suitable ***retroviral*** vector infection protocol.
AU Freie, Brian W.; *Dutt, Parmesh*; Clapp, D. Wade (1)
CS (1) Herman B. Wells Res. Cent., James Whitcomb Riley Hosp. Children, Indiana Univ. Med. Cent., Indianapolis, IN 46202 USA
SO *Cell Transplantation*, (1996) Vol. 5, No. 3, pp. 385-393.
ISSN: 0963-6897.
DT Article
LA English
AB Fanconi anemia (FA) is a complex autosomal recessive disease with *hematologic manifestations* characterized by a progressive hypoplastic anemia, hypersensitivity to clastogenic agents, and an increased incidence of acute myelogenous leukemia. The *cDNA that corrects one of four FA* complementation subtypes, named Fanconi anemia Type C (FAC) has recently been identified. We constructed a simplified recombinant ***retrovirus*** *(vMFGFAC) encoding only* the FAC cDNA, and tested its ability to correct the FAC defect in a lymphocytic cell line and primary mobilized blood progenitor cells. In addition, the *gene transfer efficiency* using a clinically applicable gene transfer protocol into normal primitive hematopoietic progenitor cells, high proliferating potential colony forming *cells (HPP-CFC), derived from* CD34+ purified cord blood cells was examined. The gene transfer efficiency was significantly enhanced when cells were ***transduced*** with supernatant *while* ***adherent*** to a 30135 KD fragment of fibronectin, FN30/35, and was similar to efficiency obtained by coculture with ***retrovirus*** packaging cells. ***Transduction*** *of an FAC deficient lymphoid* cell *line* with vMFGFAC supernatant resulted in an enhanced cell viability, and G-CSF mobilized peripheral blood cells from an FAC-deficient patient ***transduced*** *with the vMFGFAC* virus demonstrated enhanced progenitor cell colony formation. These data indicate that the vMFGFAC virus allows functional complementation of FAC in *lymphoblasts and primary* hematopoietic progenitors, and that primitive cord blood hematopoietic stem/progenitor cells can be ***transduced*** at an efficiency comparable to *protocols using cocultivation if* ***adherent*** to FN 30135 fragment.

L16 ANSWER 25 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1997:54081 BIOSIS
DN PREV199799353284
TI The baboon as an animal model for gene transfer in leukocyte ***adherence*** deficiency.
AU Bauer, T. R., Jr.; Winkler, A.; Andrews, R. G.; Hickstein, D. D.
CS VA Puget Sound Health Care System, Univ. Washington Sch. Med., Fred Hutchinson Cancer Res. Cent., Seattle, WA USA
SO Blood, (1996) Vol. 88, No. 10 SUPPL. 1 PART 1-2, pp. 276A. Meeting Info.: Thirty-eighth Annual Meeting of the American Society of Hematology Orlando, Florida, USA December 6-10, 1996 ISSN: 0006-4971.
DT Conference; Abstract; Conference
LA English

L16 ANSWER 26 OF 46 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 23
AN 1996:709500 CAPLUS
DN 126:824
TI Evaluation of the effect of ***retroviral*** gene ***transduction*** on vascular endothelial cell adhesion
AU Sackman, Jill E.; Cezeaux, Judy L.; Reddick, Tonya T.; Freeman, Michael B.; Stevens, Scott L.; Goldman, Mitchell H.
CS Medical Center, University Tennessee, Knoxville, TN, 37920, USA
SO Tissue Eng. (***1995***), 2(3), 223-234 CODEN: TIENFP; ISSN: 1076-3279
PB Liebert
DT Journal
LA English
AB Genetically modified endothelial cells (ECs) seeded on synthetic vascular grafts offer the potential to improve small diam. vascular graft patency. Despite encouraging results with naive ECs, cells ***transduced*** with ***retroviral*** vectors appear impaired in their ability to ***adhere*** to and stably colonize vascular grafts in vivo. This study addresses changes in ***retrovirally*** ***transduced*** EC adhesion as the cause of cell loss. Endothelial cells were ***retrovirally*** ***transduced*** with the bacterial neoR gene or "mock" ***transduced*** with empty viral particles. Cells were allowed to ***adhere*** to collagen IV (CIV) or fibronectin (FN) prior to exposure to 20 or 90 dyn/cm2 using a parallel plate app. Cell detachment was evaluated using time lapse videomicroscopy. Fibronectin was a significantly better adhesive protein for naive EC than CIV at both shear stresses. NeoR- ***transduced*** EC had significantly greater detachment from FN than either naive or "mock"- ***transduced*** EC. ***Transduced*** EC attachment to FN was no greater than to CIV. Flow cytometric anal. of the fibronectin receptor (FNR) showed that ***transduced*** cells have reduced receptor expression compared to naive and "mock"- ***transduced*** EC. These results indicate ***retrovirally*** ***transduced*** EC have altered FNR and adhesion to FN and that these changes may account for ***transduced*** EC loss in vivo.

L16 ANSWER 27 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
24
AN 1996:484256 BIOSIS
DN PREV199699199512
TI In vitro T lymphopoiesis: A model system for stem cell gene therapy for AIDS.
AU Rosenzweig, Michael; Marks, Douglas F.; Hempel, Donna; Johnson, R. Paul (1)
CS (1) Div. Immunol., New England Regional Primate Res. Cent., Harvard Med. Sch., One Pine Hill Drive, Southborough, MA 01772 USA
SO Journal of Medical Primatology, (1996) Vol. 25, No. 3, pp. 192-200. ISSN: 0047-2565.
DT Article
LA English
AB Stable introduction of therapeutic genes into hematopoietic stem cells has the potential to reconstitute immunity in individuals with HIV infection. However, many important questions regarding the safety and efficacy of this approach remain unanswered and may be addressed in a non-human primate model. To facilitate evaluation of expression of foreign genes in T cells derived from ***transduced*** hematopoietic progenitor cells, we have established a culture system that supports the differentiation of rhesus macaque and human CD34+ bone marrow derived cells into mature T cells. Thymic stromal monolayers were prepared from the ***adherent*** cell fraction of collagenase digested fetal or neonatal thymus. After 10-14 days, purified rhesus CD34+ bone marrow-derived cells cultured on thymic stromal monolayers yielded CD3+CD4+CD8+, CD3+CD4+CD8-, and CD3+CD4-CD8+ cells. Following stimulation with mitogens, these T cells derived from CD34+ cells could be expanded over 1,000-fold and maintained in culture for up to 20 weeks. We next evaluated the ability of rhesus CD34+ cells ***transduced*** with a ***retroviral*** vector containing the marker gene neo to undergo in vitro T cell differentiation. CD34+ cells ***transduced*** in the presence of bone marrow stroma and then cultured on rhesus thymic stroma resulted in T cells containing the ***retroviral*** marker gene. These studies should facilitate both in vitro and in vivo studies of hematopoietic stem cell therapeutic strategies for AIDS.

L16 ANSWER 28 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
25
AN 1995:510800 BIOSIS
DN PREV199598515850
TI Improved transfer of the leukocyte integrin CD18 subunit into hematopoietic cell lines by using ***retroviral*** vectors having a gibbon ape leukemia virus envelope.
AU Bauer, Thomas R., Jr.; Miller, Dusty; Hickstein, Dennis D. (1)
CS (1) Medical Service, Seattle, VA Med. Cent., 1660 S. Columbian Way, Seattle, WA 98108 USA
SO Blood, (1995) Vol. 86, No. 6, pp. 2379-2387. ISSN: 0006-4971.
DT Article
LA English
AB Leukocyte ***adherence*** deficiency (LAD) is an inherited immunodeficiency disease caused by defects in the CD18 leukocyte integrin subunit. ***Transduction*** of CD18 into hematopoietic cells from children with LAD represents a potential therapy for this disorder. In an attempt to maximize transfer and expression of CD18, we evaluated ***retroviral*** vectors with and without the neomycin selectable marker, with a modified tRNA primer binding site designed to prevent inhibition of gene expression, and with two different viral envelope proteins produced by using the amphotropic ***retrovirus*** packaging cell line PA317 or the gibbon ape leukemia virus packaging cell line PG13. The vectors were tested using ***transducing*** K562/CD11b cells and LAD Epstein-Barr virus (EBV) B cells and measuring levels of cell-surface CD11/CD18 expression by fluorescence-activated cell sorter analysis. The best results were obtained with vectors made using PG13 packaging cells, for which about 25% of the K562 cells exposed once to the vectors expressed surface CD11b/CD18 and about 25% of the LAD EBV B cells exposed three times over a 3-day period to the vectors expressed surface CD11a/CD18. In contrast, ***transduction*** of cells under similar conditions with ***retroviral*** vectors produced using PA317 producer cells yielded less than 2% of the K562 cells and less than 4% of the LAD EBV B cells expressing the CD11/CD18 heterodimer on the cell surface. The presence or absence of the neomycin resistance gene or the modified tRNA primer had no effect on CD18 gene transfer rate or expression level. The increase in ***transduction*** with PG13 vectors correlated with Northern blotting and reverse transcription-polymerase chain reaction studies that indicated that both K562 cells and the LAD EBV B cells express transcripts for the gibbon ape leukemia virus receptor at higher levels than for the amphotropic virus receptor. These findings indicate that the ***transduction*** efficiency of ***retroviral*** packaging cell lines correlates with receptor gene expression in the target cells and that vectors made using PG13 cells may be efficacious for gene therapy for LAD and other diseases in which gene transfer to hematopoietic cells is required.

L16 ANSWER 29 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
26
AN 1996:56310 BIOSIS
DN PREV199698628445
TI Effects of ***retroviral*** -mediated tissue plasminogen activator gene transfer and expression on ***adherence*** and proliferation of canine endothelial cells seeded onto expanded polytetrafluoroethylene.
AU Huber, Thomas S. (1); Welling, Theodore H.; Sarkar, Rajabrata; Messina, Louis M.; Stanley, James C.
CS (1) Sect. Vascular Surg., Dep. Surg., Univ. Florida, PO Box 100286, Gainesville, FL 32610-0286 USA
SO Journal of Vascular Surgery, (1995) Vol. 22, No. 6, pp. 795-803. ISSN: 0741-5214.
DT Article
LA English
AB Purpose: Seeding prosthetic arterial grafts with genetically modified endothelial cells (ECs) has the potential to substantially improve graft function. However, preliminary applications suggest that grafts seeded with ***retrovirally*** ***transduced*** ECs yield a significantly lower percent surface coverage than those seeded with nontransduced ECs. The objective of this study was to test the hypothesis that canine ECs ***transduced*** with the human tissue plasminogen activator (tPA) gene would have a lower rate of ***adherence*** to pretreated expanded polytetrafluoroethylene (ePTFE) both in vitro and in vivo and that they would proliferate at a slower rate on pretreated ePTFE in vitro. Methods: Early passage ECs derived from canine external jugular vein were ***transduced*** with the ***retroviral*** MFG vector containing the gene for human tPA. ECs exposed to media alone served as controls. Iodine 125-labeled ECs were seeded in vitro onto ePTFE graft segments pretreated with canine whole blood, fibronectin (50 mu-g/ml), or media alone, and the percent of ECs ***adherent*** at 1 hour were determined (n = 3). Additional tPA- ***transduced*** and -nontransduced ECs were grown for 10 days on either fibronectin (50 mu-g/ml)-pretreated ePTFE wafers or tissue culture plastic pretreated with gelatin (1%) or fibronectin (50 mu-g/ml), and the EC proliferation rates were determined (n = 3). Furthermore, 125I-labeled ECs were seeded onto fibronectin (50 mu-g/ml)-pretreated ePTFE graft segments implanted as carotid and femoral artery interposition grafts (n = 3). The grafts were harvested after 1 hour, and the percent of ECs ***adherent*** was determined. Results: Human tPA was detected by immunohistochemical staining in 61% +- 5% of the ***transduced*** ECs and was expressed at 35.4 +- 12.9 ng/hr/10-6 cells. Fibronectin and whole blood pretreatment of the ePTFE grafts led to greater EC ***adherence*** in vitro than did media alone (90.9% +- 5.3% vs 77.8% +- 5.8% vs 4.7% +- 1.1%, p ltoreq 0.05). No significant difference in the rates of ***adherence*** or proliferation was seen in vitro between the ***transduced*** and nontransduced ECs. No significant difference in proliferation was found for the ***transduced*** ECs on the three matrices tested in vitro. In contrast, ***adherence*** of the ***transduced*** ECs in vivo was significantly lower than that of nontransduced ECs (64.7% +- 2.1% vs 73.7% +- 4.1%, p ltoreq 0.05) 1 hour after implantation. Conclusions: Lower rates of surface endothelialization by genetically modified ECs in vivo do not appear to be due to an impaired capacity to initially ***adhere*** or proliferate on the synthetic graft but may result from decreased ***adherence*** after exposure to in vivo hemodynamic forces.

L16 ANSWER 30 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
27
AN 1995:535477 BIOSIS
DN PREV199598549777
TI Adhesion of human neuroblasts to HIV-1 tat.
AU Comaglia-Ferraris, P. (1); De Maria, A.; Cirillo, C.; Cara, A.; Alessandri, G.
CS (1) G. Gaslini Res. Children Hosp., 16148 Genova-Quarto Italy
SO Pediatric Research, (1995) Vol. 38, No. 5, pp. 792-796. ISSN: 0031-3998.
DT Article
LA English
AB Several neuropathologic findings in infants and children with human immunodeficiency virus type-1 (HIV-1) infection are different from those observed in adults, probably related to the fact that the ***retroviral*** infection occurs in the setting of neurodevelopment. This report describes the interaction and biologic activity of tat, the HIV-1 trans-activating protein on human neuroblasts. Two human neuroblastoma cell lines, LAN-5 and GI-CA-N, have been studied for their capability to ***adhere*** to tat (full recombinant protein) and to two different peptide residues of it. Both cells ***adhere*** to tat and tat-46-60 basic domain, although not to tat-65-80 residue, which contains the RGD (arginine-glycine-aspartic acid) motif. Adhesion to collagen I was inhibited by preincubating GI-CA-N cells with tat,46-60 although not with tat,46-60 indicating the capability of the basic residue to interfere with collagen I-induced cellular adhesion. The expression of 200-kD neurofilaments induced by collagen I was not induced by tat,46-60 indicating that neural differentiation along the same pathway is not mimicked by this peptide. Neuroblast cell proliferation was not affected

by adhesion to tat-46-60 nor to tat.65-80 GI-CA-N cells are not permissive to HIV-1 infection. However, proviral DNA was documented in the cell lysate for 14 consecutive in vitro passages, whereas HIV-1 transcription was never detectable. This would exclude the possibility that tat would be ***transduced*** by these cells. GI-CA-N stained negative for CD4, although positive for Gal-C, which may explain HIV-1 entry. Results show that immature human neural cells interact with tat protein and/or its basic residue in vitro. A mechanism similar to that herein described would possibly be active in vivo, which may help in clarifying the pathogenic mechanisms of neurologic dysfunction and destruction of the CNS observed in infants infected with HIV-1.

L16 ANSWER 31 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
28
AN 1995:511089 BIOSIS
DN PREV199598516139
TI The presence of an autologous marrow stromal cell layer increases glucocerebrosidase gene ***transduction*** of long-term culture initiating culture initiating cells (LTCICs) from the bone marrow of a patient with Gaucher disease.
AU Wells, S.; Malik, P.; Pensiero, M.; Kohn, D. B.; Nolta, J. A. (1)
CS (1) Childrens Hospital Los Angeles, Division of Research Immunology/Bone Marrow Transplantation, 4650 Sunset Boulevard, Mailstop 62, Los Angeles, CA 90027 USA
SO Gene Therapy, (1995) Vol. 2, No. 8, pp. 512-520.
ISSN: 0969-7128.
DT Article
LA English
AB Gaucher disease is a lysosmal storage disorder resulting from deficiency of the acid beta-glucosidase, glucocerebrosidase (GC). Allogeneic bone marrow transplantation has been beneficial in the treatment of Gaucher patients. Therefore, this disorder may be an ideal candidate for gene therapy by GC gene ***transduction*** of hematopoietic stem cells. We sought to increase the extent of gene transfer into CD34+ cells from the marrow of a Gaucher patient using G1GC, a simple ***retroviral*** vector containing a normal human GC cDNA. The ability of autologous stromal support and recombinant cytokines to increase the extent of ***transduction*** of colonyforming cells (CFCs) and longterm culture initiating cells (LTCICs) was assessed. The presence of a stromal layer significantly increased the extent of GC gene transfer into 14-day CFCs, as determined by polymerase chain reaction (PCR) of individual colonies (18.8% with stroma versus 5% without, P lt 0.001). Stromal support also increased the extent of ***transduction*** of LTCICs (10% with stroma versus 0.83% without, P lt 0.001). Non- ***adherent*** cells from long-term bone marrow cultures initiated with CD34+ progenitors ***transduced*** on autologous stroma had higher levels of GC enzyme activity than cultures initiated with cells ***transduced*** without stroma. The percentage of cells which were GC positive by immunohistochemistry was also increased (21.1% with stroma versus 2.7% without P = 0.0003). The addition of cytokines (IL-3, IL-6 and Steel factor) to the ***transduction*** , in the presence of stroma, significantly increased the extent of gene transfer into CFCs but not LTCICs. These studies indicate that the GC gene can be effectively ***transduced*** into LTCICs by ***retroviral*** vectors in the presence of stroma at levels significant for clinical gene therapy trials in patients with Gaucher disease.

L16 ANSWER 32 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:279588 BIOSIS
DN PREV199598293888
TI Development of ***retroviral*** vectors for use in gene therapy of leukocyte ***adherence*** deficiency.
AU Bauer, Thomas R., Jr. (1); Miller, A. Dusty; Hickstein, Dennis D.
CS (1) Med. Research Serv., Seattle Veterans Affairs Med. Center, Seattle, WA 98108 USA
SO Journal of Cellular Biochemistry Supplement, (1995) Vol. 0, No. 21A, pp. 403.
Meeting Info.: Keystone Symposium on Gene Therapy and Molecular Medicine Steamboat Springs, Colorado, USA March 26-April 1, 1995
ISSN: 0733-1959.
DT Conference
LA English

L16 ANSWER 33 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
29
AN 1995:202622 BIOSIS
DN PREV199598216922
TI Synthetic vascular grafts seeded with genetically modified endothelium in the dog: Evaluation of the effect of seeding technique and ***retroviral*** vector on cell persistence in vivo.
AU Sackman, Jill E. (1); Freeman, Michael B.; Petersen, Mark G.; Allebban, Zuhair; Niemeyer, Glenn P.; Lothrop, Clinton D., Jr.
CS (1) Dep. Surg., Univ. Tennessee Med. Cent., 1924 Alcoa Highway, Knoxville, TN 37920 USA
SO Cell Transplantation, (1995) Vol. 4, No. 2, pp. 219-235.
ISSN: 0963-6897.
DT Article
LA English
AB Unique characteristics of endothelium make it an attractive target cell for gene transfer. Genetically modified endothelial cells (ECs) seeded on synthetic vascular grafts offer the potential to control neointimal hyperplasia, decrease graft thrombogenicity and improve small diameter graft patency. This study addresses the issue of synthetic vascular graft colonization with endothelial cells ***transduced*** with noninducible ***retroviral*** marker genes in the dog. Autologous endothelial cells were enzymatically harvested and ***transduced*** with either the bacterial Neo-R gene or human growth hormone gene using ***retroviral*** vectors. All ***transduced*** cells were positive by polymerase chain reaction (PCR) amplification for the ***transduced*** gene sequence prior to graft seeding. ***Transduced*** ECs were seeded on Dacron grafts (n = 3) preclotted with autologous blood. These grafts exhibited complete endothelialization at times from 250 to 360 days. Recovered DNA, however, was negative for the ***transduced*** gene sequence when analyzed by PCR and Southern blotting. Expanded polytetrafluoroethylene (ePTFE) was evaluated (n = 8) using several different cell seeding protocols. Grafts were seeded at 3 densities (ranging from 6 times 10-3 to 1.5 times 10-5 cells/cm-2) and 2 different ***adherence*** times. Seeding substrate was also evaluated. Grafts were either preclotted with whole blood or incubated with 20 or 120 mu-g/ml fibronectin for 60 min. Graft biopsies were evaluated from 2 to 52 wk. Limited endothelialization was present in 4 dogs as early as 2 wk, but never progressed to full luminal coverage. The remaining dogs failed to ever exhibit any luminal EC ***adherence*** . Two dogs with limited EC coverage had positive DNA by PCR for the Neo-R gene sequence at 2 and 3 wk. In contrast to ***transduced*** EC's, nontransduced EC colonization of ePTFE was complete at 2 wk when seeded under conditions that ***transduced*** cells had failed to persist. Neither seeding density, ***adherence*** time, seeding substrate or ***retroviral*** vector used influenced the uniformly poor graft coverage seen with ***transduced*** cells. Results of this study indicate that despite successful gene transfer using 4 different ***retroviral*** vectors, ***transduced*** endothelial cells seeded under varying conditions appear altered in their ability to stably ***adhere*** and colonize synthetic vascular grafts in vivo.

L16 ANSWER 34 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
30
AN 1995:437295 BIOSIS
DN PREV199598451595
TI Centrifugal enhancement of ***retroviral*** mediated gene transfer.
AU Bahnson, Alfred B. (1); Dunigan, James T.; Baysal, Bora E.; Mohney, Trina; Atchison, R. Wayne; Nimgaonkar, Maya T.; Ball, Edward D.; Barranger, John A.
CS (1) Dep. Human Genetics, Graduate Sch. Public Health, Univ. Pittsburgh, Pittsburgh, PA 15261 USA
SO Journal of Virological Methods, (1995) Vol. 54, No. 2-3, pp. 131-143.
ISSN: 0166-0934.
DT Article
LA English
AB Centrifugation has been used for many years to enhance infection of cultured cells with a variety of different types of viruses, but it has only recently been demonstrated to be effective for ***retroviruses*** (Ho et al. (1993) J. Leukocyte Biol. 53, 208-212; Kotani et al. (1994) Hum. Gene Ther. 5, 19-28). Centrifugation was investigated as a means of increasing the ***transduction*** of a ***retroviral*** vector for gene transfer into cells with the potential for transplantation and engraftment in human patients suffering from genetic disease, i.e., gene therapy. It was found that centrifugation significantly increased the rate of ***transduction*** into ***adherent*** murine fibroblasts and into non- ***adherent*** human hematopoietic cells, including primary CD34+ enriched cells. The latter samples include cells capable of reconstitution of hematopoiesis in myeloablated patients. As a step toward optimization of this method, it was shown that effective ***transduction*** is: (1) achieved at room temperature; (2) directly related to time of centrifugation and to relative centrifugal force up to 10,000 g; (3) independent of volume of supernatant for volumes gtoreq 0.5 ml using non- ***adherent*** cell targets in test tubes, but dependent upon volume for coverage of ***adherent*** cell targets in flat bottom plates; and (4) inversely related to cell numbers per tube using non- ***adherent*** cells. The results support the proposal that centrifugation increases the reversible binding of virus to the cells, and together with results reported by Hodgkin et al. (Hodgkin et al. (1988) J. Virol. Methods 22, 215-230), these data support a model in which the centrifugal field counteracts forces of diffusion which lead to dissociation during the reversible phase of binding.

L16 ANSWER 35 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
31
AN 1994:438998 BIOSIS
DN PREV199497451998
TI Efficient transfer of selectable and membrane reporter genes in hematopoietic progenitor and stem cells purified from human peripheral blood.
AU Valtieri, M.; Schiro, R.; Chelucci, C.; Masella, B.; Testa, U.; Casella, I.; Montesoro, E.; Mariani, G.; Hassan, H. J.; Peschle, C. (1)
CS (1) Thomas Jefferson Cancer Inst., Thomas Jefferson Univ., Bluemle Life Sci. Build., Room 528, 233 S. 10th St., Philadelphia, PA 19107 USA
SO Cancer Research, (1994) Vol. 54, No. 16, pp. 4398-4404.
ISSN: 0008-5472.
DT Article
LA English
AB We have utilized highly purified hematopoietic progenitor and stem cells (HPCs, HSCs) from normal peripheral blood to develop methodology for: (a) efficient transfer into HPCs of a non-hematopoietic membrane reporter, i.e., the nerve growth factor receptor complementary DNA; and (b) effective gene ***transduction*** of putative HSCs, ie., cells initiating Dexter-type long-term culture (LTC-ICs). Purified HPCs induced into cycling by growth factors (interleukin 3, interleukin 6, c-kit ligand) were ***transduced*** with the N2 ***retroviral*** vector containing the neomycin resistance (neo-r) gene. More than 80% of ***transduced*** HPCs were resistant to the toxic G418 level. Thereafter, the HPCs were effectively ***transduced*** with the LNSN ***retroviral*** vector containing a nerve growth factor receptor complementary DNA; the nerve growth factor receptor was detected on gtoreq 18% of the ***transduced*** RPCs. These experiments provide a new tool from which (a) to monitor expression of a ***transduced*** membrane reporter on hematopoietic cells, particularly at the level of HPCs/HSCs, and (b) to characterize the ***transduced*** cells by double- and triple-labeling membrane antigen analysis. Purified HPCs/HSCs grown in Dexter-type LTC were ***transduced*** at 1 week by exposure to supernatant N2 ***retroviral*** particles in the absence of exogenous hematopoietic growth factors. The procedure, devoid of toxic effects, allowed an efficient neo-r ***transduction*** into LTC-ICs. Thus, we consistently detected neomycin-resistant mRNA in the clonal progeny of HPCs produced in LTC at 5-8 weeks in both the nonadherent and ***adherent*** fractions; this timing of expression coincides with that of HPC production by LTC-ICs, thereby indicating the effective ***transduction*** of the LTC-ICs. These experiments represent a first step toward development of preclinical models for gene transfer into human peripheral blood HSCs by complex ***retroviral*** vectors.

L16 ANSWER 36 OF 46 CAPLUS COPYRIGHT 2002 ACS
AN 1995:180279 CAPLUS
DN 122:257320
TI ***Transduction*** of human bone marrow by adenoviral vector
AU Mitani, Kohnoske; Graham, Frank L.; Caskey, C. Thomas
CS Howard Hughes Medical Institute, Baylor College Medicine, Houston, TX, 77030, USA
SO Hum. Gene Ther. (***1994***), 5(8), 941-8
CODEN: HGTHE3; ISSN: 1043-0342
DT Journal

LA English
AB Recombinant adenoviral vectors have been shown to be potential new tools for a variety of human gene therapy protocols. The authors examd. the effectiveness of an adenovirus vector for gene transfer into human bone marrow (BM). Mononuclear cells from one adenosine deaminase (ADA)-deficient and two normal human BM samples were ***transduced*** by an E1-defective adenoviral vector encoding human ADA and kept in myeloid long-term culture. ***Retroviral*** gene transfer was also performed with the ADA-deficient bone marrow as a control. The ***transduced*** cells were harvested at different times and the expression of the vector-encoded ADA in crude cell exts. of non-***adherent*** cells was analyzed. The expression from Ad-ADA was higher than that from a ***retroviral*** vector at 1 wk post-***transduction*** . In half of the expts., the ADA activity decreased with passage. Unexpectedly, sustained expression from Ad-ADA was obsd. in the other half. At the end of the expts. (2 mo), free virus from BM cultures which showed sustained expression of ADA was detected on 293 cells. Several independent virus clones were isolated and analyzed and found to be Ad-ADA. The results suggest potential use of adenoviral vectors for gene therapy that does not require sustained expression, as with cytokine gene transfer for cancer therapy. However, the finding that infectious virus can sometimes persist might raise issues regarding the leakiness of human adenovirus vectors in cells of some human tissues.

L16 ANSWER 37 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:55753 BIOSIS
DN PREV199598070053
TI CD34+++ stem/progenitor cells purified from cryopreserved cord blood can be ***transduced*** with high efficiency as a ***retroviral*** vector and expanded ex vivo with stable integration and expression of Fanconi anemia complement C gene.
AU Lu, L.; Ge, Y.; Li, Z.-H.; Freie, B.; Clapp, D. W.; Broxmeyer, H. E.
CS Indiana Univ. Sch. Med., Indianapolis, IN USA
SO Blood, (1994) Vol. 84, No. 10 SUPPL. 1, pp. 355A.
Meeting Info.: Abstracts Submitted to the 36th Annual Meeting of the American Society of Hematology Nashville, Tennessee, USA December 2-6, 1994
ISSN: 0006-4971.
DT Conference
LA English

L16 ANSWER 38 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
32
AN 1993:525501 BIOSIS
DN PREV199396138908
TI Increased sensitivity to TNF-mediated cytotoxicity of BL6 melanoma cells after H-2K-b gene transfection.
AU Kim, Misoon; Herberman, Ronald; Gorelik, Elieser (1)
CS (1) Pittsburgh Cancer Inst., Biomedical Sci. Tower, Room W954, DeSoto and O'Hara Street, Pittsburgh, PA 15213 USA
SO Journal of Immunology, (1993) Vol. 151, No. 7, pp. 3467-3477.
ISSN: 0022-1767.
DT Article
LA English
AB Transfection of the H-2K-b and neo-r genes into BL6-8 (H-2K-b-, H-2D-b+) melanoma clone resulted in various phenotypic changes with appearance of soybean agglutinin (SBA) and Grifonia Simplicifolia 1-B-4 (GS1B-4) lectin binding carbohydrates and loss of melanoma-associated antigen (MAA). In parallel H-2K-b gene-transfected melanoma cells showed increased sensitivity to TNF lysis. To further delineate the ability of H-2K-b gene to induce the phenotypic changes and TNF sensitivity, BL6-8 melanoma clone was transfected with the H-2K-b gene alone without cotransfection with neo-r gene and transfected cells were selected for ***adherence*** to SBA lectin-conjugated agarose beads. Analysis of isolated clones revealed that 38 of 47 tested clones have been found to be expressing the H-2K-b parallel these cells became sensitive to TNF lysis. Although all clones with high expression of H-2K-b Ag were sensitive to TNF lysis, it seems unlikely that H-2K molecules are directly required for or involved in TNF-induced melanoma cell lysis. This conclusion is based on findings that four H-2K-b-transfected clones selected on SBA agarose beads did not expressed H-2K-b Ag but manifested increase in SBA and GSI B4 lectin binding and loss of MAA and also became sensitive to TNF lysis. It seems that increase in TNF sensitivity is a part of the broad phenotypic changes induced by the H-2K -gene that remained stable even in the clones in which the transfected H-2K-b gene was lost or down-regulated. We believe that the effects of the H-2K-b gene on melanoma cell phenotype and TNF sensitivity are indirect and are probably mediated via its inhibition of the melanoma-associated ecotropic ***retrovirus*** production and activation of some repressed cellular genes. Study of the mechanisms responsible for TNF sensitivity of BL6 melanoma cells revealed that the H-2K-b gene transfection resulted in an increase in p55 TNF receptor expression. TNF-induced activation of phospholipase A-2 and release of arachidonic acid metabolites was observed only in the H-2K-b transfected, but not in BL6-8 melanoma cells transfected with neo-or class II H-21A-k genes. TNF resistance of BL6 melanoma cells appeared to be due to a block in ***transduction*** of the lytic signal that was reversed after transfection with H-2K-b gene.

L16 ANSWER 39 OF 46 CAPLUS COPYRIGHT 2002 ACS
AN 1993:618398 CAPLUS
DN 119:218398
TI Cytokine gene transfer into tumor cells and its application to human cancer
AU Rosenthal, Felicia M.; Cronin, Kathryn; Guarini, Rita; Gansbacher, Bernd
CS Dep. Hematol. Oncol., Memorial Sloan Kettering Cancer Cent., New York, NY, 10021, USA
SO Prog. Immunol., Vol. VIII, Proc. Int. Congr. Immunol., 8th (***1993***), Meeting Date 1992, 381-7. Editor(s): Gergely, Janos. Publisher: Springer, Berlin, Germany.
CODEN: 59JMA5
DT Conference; General Review
LA English
AB A review with 39 refs. Introduction of genes encoding cytokines into tumor cells induces constitutive local secretion of the cytokine at the site where effector cells encounter theer target. Thus, cytotoxic effector cells at a tumor site will get activated and enriched in no. Of all gene transfer techniques, ***retroviral*** mediated gene therapy is the most suitable approach for ***transducing*** genes into cells for clin. use. This technique affords stable integration into cellular DNA and a broad host range and makes the infection of ***adherent*** cells as well as suspension cells including lymphoid, myeloid and hematopoietic stem cells possible. Cytokine gene transfer in the murine and human system are discussed.

L16 ANSWER 40 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
33
AN 1993:137062 BIOSIS
DN PREV199395069862
TI Factors affecting the ***transduction*** of pluripotent hematopoietic stem cells: Long-term expression of a human adenosine deaminase gene in mice.
AU Einerhand, M. P. W.; Bakx, T. A.; Kukler, A.; Valerio, D. (1)
CS (1) Inst. Appl. Radiobiol. Immunol. TNO, PO Box 5815, 2280 HV Rijswijk Netherlands Antilles
SO Blood, (1993) Vol. 81, No. 1, pp. 254-263.
ISSN: 0006-4971.
DT Article
LA English
AB An amphotropic ***retroviral*** vector, LgAL(DELTA-Mo + PyF101) containing a human adenosine deaminase (ADA) cDNA was used to optimize procedures for the lasting genetic modification of the hematopoietic system of mice. The highest number of ***retrovirally*** infected cells in the hematopoietic tissues of long-term reconstituted mice was observed after transplantation of bone marrow (BW) cells that had been cocultured in the presence of both interleukin-1-alpha (IL-1-alpha) and IL-3. A significantly lower number was detected when IL-1-alpha was omitted from such cocultures. The yield of cells that generate spleen colony-forming cells (CFU-S) in the BM of lethally irradiated recipients (MRA-CFU-S) significantly improved on inclusion of the ***adherent*** cell fraction of cocultures in the transplant. ***Retroviral*** integration patterns in MRA-CFU-S-derived spleen colonies showed that an MRA-CUF-S can produce many CFU-S during BM regeneration. Expression of hADA was detected in the circulating white blood cells of long-term reconstituted animals, demonstrating that the LgAL(DELTA-Mo + PyF101) vector is capable of directing the sustained expression of hADA, and in approximately 35% of the ***transduced*** MRA-CFU-S-derived spleen colonies. These results should facilitate the development of gene therapy protocols for the treatment of severe combined immunodeficiency caused by a lack of functional ADA.

L16 ANSWER 41 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:321565 BIOSIS
DN PREV199396029915
TI Elevated levels of heme oxygenase-1 activity and mRNA in peripheral blood ***adherent*** cells of acquired immunodeficiency syndrome patients.
AU Levere, Richard D.; Staudinger, Robert; Loewy, Gabriel; Kappas, Attallah; Shibahara, Shigeki; Abraham, Nader G.
CS Dep. Med., New York Med. College, Valhalla, NY 10595 USA
SO American Journal of Hematology, (1993) Vol. 43, No. 1, pp. 19-23.
ISSN: 0361-8609.
DT Article
LA English
AB Patients with the acquired immunodeficiency syndrome (AIDS) commonly develop hematological abnormalities, including anemia, leukopenia, and thrombocytopenia. Heme synthesis and heme degradation are critical to the maintenance of cellular heme homeostatis and to hematopoietic differentiation. We examined heme oxygenase activity and expression of the heme oxygenase gene in ***adherent*** cells (monocytes-macrophages) obtained from the peripheral blood of AIDS patients and normal controls. Heme oxygenase activity in normal control cells was 43+-16 pmol bilirubin formed/4 times 10-5 cells/hr as compared to 133+-30 pmol bilirubin formed/4 times 10-5 cells/hr in the AIDS patients. Via blot hybridization analysis with human heme oxygenase cDNA, heme oxygenase mRNA levels in cells of the normal and the AIDS patients were compared. Total RNA from normal cells displayed only weak hybridization with the cDNA probe. In contrast, cells from peripheral blood of the AIDS patients displayed marked increases over normal levels in heme oxygenase mRNA. Heme oxygenase activity could be substantially suppressed by the competitive inhibitor of the enzyme, Sn-mesoporphyrin. Elevated heme oxygenase activity in cells of AIDS patients could produce a decrease in cellular heme needed for ***transductional*** signalling for the growth factor network, which regulates the hematopoietic microenvironment, and for other metabolic purposes. Suppression of heme catabolism by inhibitors of this enzyme may thus be useful in potentiating erythropoietic responses in this disorder.

L16 ANSWER 42 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1994:251566 BIOSIS
DN PREV199497264566
TI Potential of ***retrovirally*** marked stem hematopoietic cells: Relevance to stimulation by growth factors.
AU Chertkov, I. L. (1); Abrakham, Neder G.
CS (1) Hematol. Sci. Cent., Acad. Med. Sci. Russ., Moscow Russia
SO Gematologiya i Transfuziologiya, (1993) Vol. 38, No. 7, pp. 8-14.
ISSN: 0234-5730.
DT Article
LA Russian
SL English
AB Lethally irradiated mice were reconstituted with hematopoietic cells ***retrovirally*** marked by human ADA sequence. Before and during gene transfer adult bone marrow cells were prestimulated by a combination of exogenous growth factors, IL-6 and kit-ligand, or by culture on irradiated ***adherent*** cell layer of long-term bone marrow culture. Twelve-day-old embryonic liver cells were ***transduced*** without prestimulation with exogenous growth factors. In mice reconstituted with growth factors stimulated adult bone marrow cells during 4 months after transplantation 200-300 hematopoietic cell clones were functioning simultaneously. Five months and later after reconstitution oligo-monoclonal hematopoiesis was revealed. The findings suggest that growth factors induce long-lasting proliferation of quiescent pHSC as a result of which clone(s) with proliferative advantage replace all others and only this clone(s) persists during long time, up to 11 months. Vice versa, in mice reconstituted with adult or embryonic hematopoietic cells which were ***transduced*** without growth factors prestimulation, the phase of polyclonal hematopoiesis was never observed and hematopoietic cell clonal succession was revealed. The data obtained for the first time demonstrate artifactual influence of high-concentration IL-6 and kit-ligand on the developmental potential of hematopoietic stem cell. The model can be useful for the study of mechanism of hematopoiesis regulation, proliferative and developmental potential of primitive HSC and growth factors effect on them.

L16 ANSWER 43 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
34
AN 1992:185487 BIOSIS
DN BA93:98437
TI GENETICALLY ENGINEERED ENDOTHELIAL CELLS REMAIN ***ADHERENT*** AND VIABLE AFTER STENT DEPLOYMENT AND EXPOSURE TO FLOW IN-VITRO.
AU FLUGELMAN M Y; VIRMANI R; LEON M B; BOWMAN R L; DICHEK D A
CS BUILDING 10, ROOM 7D-18, NATL. INST. HEALTH, BESTHESDA, MD. 20892.
SO CIRC RES, (1992) 70 (2), 348-354.
CODEN: CIRUAL. ISSN: 0009-7330.
FS BA; OLD
LA English
AB Intravascular stents, currently in experimental human use for recurrent arterial stenosis, are plagued by subacute thrombosis. As a therapeutic approach to stent-related thrombosis, we and others have suggested coating stents with endothelial cells before implantation. In a previous study we demonstrated the feasibility of coating stents with endothelial cells that were genetically modified to secrete large amounts of human tissue plasminogen activator. In the present study we attempted both to develop a clinically applicable protocol for stent seeding and to test whether seeded cells would remain ***adherent*** to stents after exposure to pulsatile flow. Endothelial cells were harvested from the saphenous veins of sheep with survival of the donor animals. Harvested cells were ***transduced*** with a ***retroviral*** vector containing a marker gene and seeded onto catheter-mounted stents under sterile conditions. Scanning electron microscopy revealed complete coverage of the stent surfaces by seeded cells. Stents were expanded and exposed to pulsatile flow in vitro. Substantial cell retention was observed on the lateral stent surfaces by light microscopy and scanning electron microscopy; fewer cells were seen on the luminal and abluminal surfaces. Removal of seeded cells from flow-exposed stents by trypsin digestion resulted in the recovery of approximately 70% of the seeded cells. These cells were viable and healthy as judged by their ability to proliferate to confluence with the same kinetics as control (non-flow-exposed) cells. Autologous genetically modified endothelial cells can be seeded onto catheter-mounted stents in a sterile manner, and stent deployment under flow conditions results in substantial retention of viable cells.

L16 ANSWER 44 OF 46 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 92220145 EMBASE
DN 1992220145
TI Gene therapy model for stromal precursor cells of hematopoietic microenvironment.
AU Drize N.J.; Surin V.L.; Gan O.I.; Deryugina E.I.; Chertkov J.L.
CS National Research Center, Hematology, Novo-Zykovsky 4a,125167 Moscow, Russia, Russia
SO Leukemia, (1992) 6/SUPPL. 3 (174S-175S).
ISSN: 0887-6924 CODEN: LEUKED
CY United Kingdom
DT Journal; Conference Article
FS 004 Microbiology
022 Human Genetics
025 Hematology
LA English
SL English
AB Marker bacterial Neo(r) gene was ***transduced*** by ***retroviral*** gene transfer into stromal precursor cells making up the hematopoietic microenvironment in murine long-term bone marrow cultures (LTBMC). Cultures were infected six times during the first 3 weeks of cultivation. At 4 weeks, the ***adherent*** cell layers (ACLs) were implanted under the renal capsule of syngeneic unirradiated and irradiated mice. Cells from newly formed ectopic foci were explanted into secondary LTBMC. ACLs containing the marker gene were detected by polymerase chain reaction. About 74% of stromal cells in ACLs contained Neo(r) gene. The possibility of stable gene ***transduction*** into stromal precursor cells competent to transfer the hematopoietic microenvironment was established.

L16 ANSWER 45 OF 46 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 90208556 EMBASE
DN 1990208556
TI Correction of CD18-deficient lymphocytes by ***retrovirus*** -mediated gene transfer.
AU Wilson J.M.; Ping A.J.; Krauss J.C.; Mayo-Bond L.; Rogers C.E.; Anderson D.C.; Todd III R.F.
CS Howard Hughes Med. Institute, Dept. of Internal Medicine, Univ. of Michigan Med. Sch.,Ann Arbor, MI 48109-0650, United States
SO Science, (1990) 248/4961 (1413-1416).
ISSN: 0036-8075 CODEN: SCIEAS
CY United States
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
047 Virology
LA English
SL English
AB Leukocyte adhesion deficiency (LAD) is an inherited disorder of leukocyte function caused by derangements in CD18 expression. The genetic and functional abnormalities in a lymphocyte cell line from a patient with LAD have been corrected by ***retrovirus*** -mediated ***transduction*** of a functional CD18 gene. Lymphocytes from patients with LAD were exposed to CD18-expressing ***retrovirus*** and enriched for cells that express CD11a and CD18 (LFA-1) on the cell surface. Molecular and functional analyses of these cells revealed (i) one copy of proviral sequence per cell, (ii) viral-directed CD18 RNA that exceeded normal endogenous levels, (iii) normal quantities of CD11a and CD18 protein on the cell surface, and (iv) reconstitution of LFA-1-dependent adhesive function.

L16 ANSWER 46 OF 46 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
35
AN 1990:28313 BIOSIS
DN BA89:16279
TI BINDING OF RADIATION LEUKEMIA VIRUSES TO A THYMIC LYMPHOMA INVOLVES SOME
CLASS I MOLECULES ON THE T CELL AS WELL AS THE T CELL RECEPTOR COMPLEX.
AU O'NEILL H C
CS DEP. EXP. PATHOL., JOHN CURTIN SCH. MED. RES., AUST. NATL. UNIV., CANBERRA, ACT 2601, AUST.
SO JMCI (J MOL CELL IMMUNOL), (1989) 4 (4), 213-224.
CODEN: JMCIDI. ISSN: 0724-6803.
FS BA; OLD
LA English
AB Radiation leukemia virus (RadLV)-induced thymomas and malignant thymocytes from AKR mice have been shown to bind specifically ***retrovirus*** produced by these cell lines. Each lymphoma has been shown to have greatest specificity for cognate virus suggestive of an immune-specific receptor. The question of receptor identity has been addressed here using the RadLV-induced murine T cell lymphoma, C6VL/1, and antibodies specific for known cell surface determinants present on these cells. This lymphoma has been shown to bind both homologous and heterologous RadLV isolates, but to have greatest specificity for homologous ***retrovirus*** since homologous free virions can best block the interaction between cells and virus ***adhered*** to the wells of a microtitre plate. A clonotypic anti-TCR antibody has been shown to completely inhibit C6VL/1 binding to the homolgous virus, RadLV/C6VL, but not to the heterologous virus, RadLV/VL3. Anti-CD4, anti-Thy1.2 as well as anti-H-2Kb and not anti-H2Db antibodies were found to partially inhibit the interaction with both RadLV/C6VL and RadLV/VL3, yet neither of these virus preparations appears to be contaminated with Class I molecules as measured by radioimmunoassay. The binding interaction between C6VL/1 and RadLV/C6VL appears specifically to involve the TCR since antibody against the clonotypic site on the TCR heterodimer uniquely inhibits this interaction, while the binding of C6VL/1 to RadLV/VL3 appears to involve the H-2Kb molecule. When free virus particles were absorbed to receptors on C6VL/1, both RadLV/VL3 and RadLV/C6VL inhibited the binding of antibody to the TCR and CD4 molecules, while the binding of several anti-H-2Kb antibodies was specifically inhibited by RadLV/VL3. There are at least two known T cell surface structures involved in the interaction of the T cell lymphoma, C6VL/1, with RadLV. These are the TCR complex (comprising the TCR heterodimer and CD4), and the Class I H-2Kb molecule. Since the TCR molecule has been shown to comodulate with H-2Kb molecules when cells were cultured in the presence of anti-H-2Kb antibodies, and the CD4 and H-2kb molecules have been shown to comodulate with the TCR on only a subpopulation of C6VL/1 cells treated with anti-TCR antibody, this suggests that the H-2Kb molecule may also be part of the larger molecule complex including CD4/8 which can form around the TCR heterodimer. Since antibody to the H-2Kb molecule can also inhibit proliferation of these cells, the possibility exists that at least some H-2Kb molecules may be linked to the signal ***transducing*** mechanism on the cell which includes the TCR and CD3 complex.

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=> s lentivir? and pre-stimulat? and stem cell and fibronectin
L1 0 LENTIVIR? AND PRE-STIMULAT? AND STEM CELL AND FIBRONECTIN

=> s lentivir? and pre-stimulat? and stem cell
L2 2 LENTIVIR? AND PRE-STIMULAT? AND STEM CELL

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L2 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:311858 BIOSIS
DN PREV200100311858
TI Characterization of murine bone marrow side-population (SP) cells: Implication for gene transduction.
AU Yamada, Kaoru (1); Walsh, Christopher E. (1)
CS (1) Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 516a-517a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB A variety of methods are used to identify and isolate hematopoietic stem cells the target cells for gene transfer of inherited hematologic disorders. The technique utilizing Hoechst dye efflux identifies a "side population" (SP) fraction capable of hematopoietic reconstitution and radioprotection in mice. In this study we further characterized murine SP cells for their use as targets for gene transfer. We isolated SP cells from C57B1/6 mice bone marrow and examined cell cycle kinetics (n=3). Approximately 90% of freshly isolated SP cells resided in G0/G1, 10% in S, and <1% in G2/M phase of the cell cycle. We then analyzed murine SP cells for clonogenicity with methylcellulose assay. Single SP cells were sorted into 96 well plates containing methylcellulose and cytokines. At 1 week of incubation, 48% (274/596) of wells containing mIL-3, hIL-6, and mSCF produced mixed colonies (granulocyte, monocyte, megakaryocyte, and stromal fibroblast) and 3% (11/384) of wells containing mIL-7 produced B-cell colonies. When 30 SP cells/well were cultured with mIL-3, hIL-6, mSCF, and hFlt3-ligand, a 3-log multi-lineage expansion was measured after 10 days. The capacity for multi-lineage expansion is implicating as ***stem*** ***cell*** . Based on this data, we then set out to examine whether a ***lentiviral*** vector would transduce murine SP cells due to the ability of ***lentiviral*** vectors to transduce quiescent cells. A VSV-G pseudeo-typed self-inactivating ***lentiviral*** vector carrying EGFP cDNA (SIN/EGFP) was constructed. In vitro transduction of murine SP cells was performed in both the presence and absence of cytokines (overnight or 2 days ***pre*** - ***stimulation*** and 2 days transduction) at an MOI of >100. In the absence of cytokines 30% of colonies were positive for EGFP; in the presence of cytokines 100% of colonies were EGFP + (measured by flow cytometry). EGFP fluorescence of individual colonies was remarkable for a variegated fluorescence pattern. We then isolated SP cells from Ly5.2 mice transduced with SIN/EGFP vector at an MOI of >100 without cytokine stimulation. 200 cells were transplanted into lethal-irradiated Ly5.1 recipient mice. Five of the 6 mice engrafted and at two months post-transplantation 50% of the peripheral blood cells expressed donor cell phenotype. Of the Ly5.2 donor cells, 10% were EGFP positive. Experiments of viral transduction of SP cells using cytokine stimulation are on going. In summary, our data suggests that murine SP cells are quiescent, capable of hematopoietic reconstitution and amenable to gene transfer using ***lentiviral*** vectors.

L2 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 2001:676635 CAPLUS
DN 135:236383
TI Highly efficient gene transfer into human repopulating stem cells by RD114 envelope protein pseudotyped retroviral vector particles which pre-adsorb on retronectin-coated plates
IN Kelly, Patrick F.; Vanin, Elio F.
PA St. Jude Children's Research Hospital, USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1
PATENT NO. KIND DATE APPLICATION NO. DATE
PI WO 2001066150 A2 20010913 WO 2001-US7212 20010307
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
US 2001051375 A1 20011213 US 2001-801302 20010307
PRAI US 2000-187534 P 20000307
AB The present invention relates to a method for efficiently introducing exogenous genes into stem cells, particularly human stem cells. The method optionally includes the steps of inducing the proliferation of target cells by ***pre*** - ***stimulation*** with cytokines and/or growth factors, followed by incubating these cells with RD114-pseudotyped vector particles. In a specific embodiment, the vector particles are retronectin-immobilized or ultracentrifugation-concd. retroviral vector particles pseudotyped with the feline endogenous retrovirus (RD114) envelope protein. The present invention further discloses a method for somatic gene therapy, which can be used for various therapeutic applications and involves introducing a gene of interest contained within the retroviral genome into human repopulating stem cells followed by introducing these cells into a human host. Finally, the present invention discloses a method for monitoring the efficiency of the ***stem*** ***cell*** -mediated gene transfer based on detecting the presence of the genes (or the expression products) of the retroviral vector in various ***stem*** ***cell*** -derived lineages of the host.

=> s lentivir? and stem cell
L3 195 LENTIVIR? AND STEM CELL

=> s l3 and fibronectin
L4 8 L3 AND FIBRONECTIN

=> dup rem l4
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L5 6 DUP REM L4 (2 DUPLICATES REMOVED)

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 6 ANSWERS - CONTINUE? Y/(N):y

L5 ANSWER 1 OF 6 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2001201966 EMBASE
TI ***Fibronectin*** fragment CH-296 inhibits apoptosis and enhances ex vivo gene transfer by murine retrovirus and human ***lentivirus*** vectors independent of viral tropism in nonhuman primate CD34(+) cells.
AU Donahue R.E.; Sorrentino B.P.; Hawley R.G.; Sung An D.; Chen I.S.Y.; Wersto R.P.
CS R.P. Wersto, Flow Cytometry Unit, Gerotology Research Center, National Institute on Aging, 5600 Nathan Shock Drive, Baltimore, MD 21224, United States. Werstor@grc.nia.nih.gov
SO Molecular Therapy, (2001) 3/3 (359-367).
Refs: 57
ISSN: 1525-0016 CODEN: MTOHCK
CY United States
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LA English
SL English
AB The ***fibronectin*** fragment CH-296 improved gene transfer to cytokine-mobilized nonhuman primate CD34(+) cells irrespective of tropism to the MoMLV, GaLV, and VSV-G envelope proteins using murine ***stem*** ***cell*** virus (MSCV) and human immunodeficiency virus-1 (HIV-1)-based retrovirus vectors. For the HIV-1 ***lentivirus*** vector, CH-296 enhanced gene transfer in the absence of added hematopoietic growth factors necessary for ex vivo ***stem*** ***cell*** expansion. In the presence of CH-296, apoptosis of CD34(+) cells was inhibited, and in mobilized peripheral blood CD34(+) cells, cell division was stimulated as measured by cell history/tracking experiments.

L5 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2001:138150 BIOSIS
DN PREV200100138150
TI Gene transfer into nonhuman primate hematopoietic stem cells: Implications for gene therapy.
AU Hanazono, Yutaka (1); Terao, Keiji; Ozawa, Keiya
CS (1) Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi, Kawachi, Tochigi, 329-0498: hanazono@jichi.ac.jp Japan
SO Stem Cells (Miamisburg), (2001) Vol. 19, No. 1, pp. 12-23. print.
ISSN: 1066-5099.
DT General Review
LA English
SL English
AB Hematopoietic stem cells (HSCs) are desirable targets for gene therapy because of their self-renewal and multilineage differentiation abilities. Retroviral vectors are extensively used for HSC gene therapy. However, the initial human trials of HSC gene marking and therapy showed that the gene transfer efficiency into human HSCs with retroviral vectors was very low in contrast to the much higher efficiency observed in murine experiments. The more quiescent nature of human HSCs and the lower density of retroviral receptors on them hindered the efficient gene transfer with retroviral vectors. Since nonhuman primates have marked similarity to humans in all aspects including the HSC biology, their models are considered to be important to evaluate and improve gene transfer into human HSCs. Using these models, clinically relevant levels (around 10% or even more) of gene-modified cells in peripheral blood have recently been achieved after gene transfer into HSCs and their autologous transplantation. This has been made possible by improving ex vivo transduction conditions such as introduction of Flt-3 ligand and specific ***fibronectin*** fragment (CH-296) into ex vivo culture during transduction, and the use of retroviral vectors pseudotyped with the gibbon ape leukemia virus or feline endogenous retrovirus envelope. Other strategies including the use of ***lentiviral*** vectors and in vivo selective expansion of gene-modified cells with the drug resistance gene or selective amplifier gene (also designated the molecular growth switch) are now being tested to further increase the fraction of gene-modified

cells using nonhuman primate models. In addition to the high gene transfer efficiency, high-level and long-term expression of transgenes in human HSCs and their progeny is also required for effective HSC gene therapy. For this purpose, other backbones of retroviral vectors such as the murine ***stem*** ***cell*** virus and cis-DNA elements, such as the beta-globin locus control region and the chromatin insulator, also need to be tested in nonhuman primate models. Nonhuman primate studies will continue to provide an important framework for human HSC gene therapy. Well-designed nonhuman primate studies will also offer unique insights into the HSCs, immune system, and transplantation biology characteristic of large animals.

L5 ANSWER 3 OF 6 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2000117373 EMBASE
TI Gene transfer into stimulated and unstimulated T lymphocytes by HIV-1-derived ***lentiviral*** vectors.
AU Costello E.; Munoz M.; Buetti E.; Meylan P.R.A.; Diggelmann H.; Thali M.
CS E. Costello, Department of Surgery, Royal Liverpool Univ. Hosp., 5th Floor UCD Building, Daulby Street, Liverpool L69 3GA, United Kingdom
SO Gene Therapy, (2000) 7/7 (596-604).
Refs: 37
ISSN: 0969-7128 CODEN: GETHEC
CY United Kingdom
DT Journal; Article
FS 022 Human Genetics
LA English
SL English
AB Genetic modification of T lymphocytes holds great potential for treatments of cancer, T cell disorders and AIDS. While in the past recombinant murine retroviruses were the vectors of choice for gene delivery to T cells, vectors based on ***lentiviruses*** can provide additional benefits. Here, we show that VSV-G pseudotyped HIV 1 vector particles delivering the enhanced green fluorescent protein (EGFP) efficiently transduce human T lymphocytes. Transduction efficiency was optimal when infection included centrifugation of cells with concentrated vector supernatant in the presence of Polybrene. In contrast to previous reports describing murine retrovirus-mediated gene transfer to T lymphocytes, ***fibronectin*** did not improve the transduction efficiency of the VSVG-pseudotyped HIV-1 particles. Similar gene transfer efficiencies were observed following stimulation of cells with PHA/IL-2 or anti-CD3i/CD28i antibodies, although greater transgene expression was observed in the latter case. Interestingly, production of vectors in the absence of the accessory proteins Vif, Vpr, Vpu and Nef was accompanied by a 50% decrease in transduction efficiency in activated T cells. Transduction of T cells that were not stimulated before infection was achieved. No transduction of non-prestimulated cells was observed with a GALV-pseudotyped murine retroviral vector The requirement for accessory proteins in nonprestimulated cells was more pronounced. Our results have implications for ***lentiviral*** vector targeting of other cells of the hematopoietic system including stem cells.

L5 ANSWER 4 OF 6 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:311452 BIOSIS
DN PREV200100311452
TI VSV-G pseudotyped feline immune deficiency virus (FIV) vectors are expressed in K562 cells but not in other leukemic cell lines or primary CD34+ cells.
AU Laufs, S. (1); Gentner, B. (1); Zeller, W. J. (1); Sauter, S. L.; Ho, A. D.; Fruehauf, S.
CS (1) German Cancer Research Center, D0200, Heidelberg Germany
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 2, pp. 381b. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB HIV-1 based ***lentiviral*** vectors efficiently transduce non-dividing cells, but present complex safety concerns. Non-primate feline ***lentiviruses*** might provide safer alternatives. Pseudotyping with the vesicular stomatitis virus G-glycoprotein (VSV-G) allows receptor independent target cell transduction. Recently developed FIV vectors contain the human cytomegalovirus (CMV) immediate early gene promotor in place of the entire FIV long-terminal repeat U3 region. We were interested in the transduction of human hematopoietic cell lines and of human CD34+ peripheral blood progenitor cells (PBPC) as this has not been reported so far and as PBPC are a possible target population for FIV vector-based gene therapy. First generation FIV vectors (pCFIV) and a construct of second generation FIV vectors (deletion of the vif and orf (pCFIVDELTAorfDELTAvif) accessory genes), both expressing the enhanced green flourescent protein (EGFP) were produced with a three-plasmid vector system and titers of 7.8X105 colony forming units (cfu)/ml on HT1080 cells for pCFIV and 4.2X106 cfu/ml for pCFIVDELTAorfDELTAvif were obtained after concentration by ultrafiltration. The leukemia cell lines K562, SD-1, BV173 and KG1A were incubated with either concentrated or unconcentrated viral supernatants for up to 48 hours in ***fibronectin*** (FN; fragment CH-296)-coated or uncoated plates +- polybrene with a multiplicity of infection of up to 10. FACS analysis of cells transduced with the original pCFIV vector or with the pCFIVDELTAorfDELTAvif showed EGFP expression K562 cells of up to 83.8% +- 2.25% for pCFIV and 88.7% +- 2.8% for pCFIVDELTAorfDELTAvif while KG1A, SD-1, and BV173 cells failed to express EGFP above background levels after transduction with either vector. Next we transduced CD34+ selected fresh or cryopreserved/thawed PBPC from six different tumor patients for 24-48h +- cytokines (Flt-3 ligand/thrombopoietin/ ***stem*** ***cell*** factor, FLTS), +- FN, +- polybrene. Interestingly, we could not detect EGFP expression above background levels in the transduced PBPC in any sample (EGFP+:0.6% +- 0.4). As control vector we used a murine retroviral vector supernatant for 48 h +FLTS +FN on CD34+ PBPC (n=6 patients) which resulted in a transgene expression of 5.3 % +- 2.8 in the progeny of transduced cells. Our data clearly show that FIV vector processing and expression is enhanced in K562 cells while normal CD34+ PBPC or other leucemic cell lines do not appear to be suitable targets for FIV vectors.

L5 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2002 ACS
AN 2000:452060 CAPLUS
DN 133:329002
TI Basic studies toward hematopoietic ***stem*** ***cell*** gene therapy
AU Hanazono, Yutaka; Dunbar, Cynthia E.; Donahue, Robert E.; Kato, Ikunoshin; Ueda, Yasuji; Hasegawa, Mamoru; Urabe, Masashi; Kume, Akihiro; Terao, Keiji; Ozawa, Keiya
CS Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical School, Tochigi, 329-0498, Japan
SO Keio Univ. Symp. Life Sci. Med. (2000), 5(Cell Therapy), 159-169
CODEN: KUSMF9
PB Springer-Verlag Tokyo
DT Journal; General Review
LA English
AB A review with 40 refs. Hematopoietic stem cells (HSCs), because they have a self-renewal ability and can generate progeny of all kinds of blood cells throughout one's life, are an ideal target for gene therapy. Retroviral vectors are predominantly used for transduction of HSCs, but the gene transfer efficiency is extremely low. Several efforts have been made at achieving clin. relevant gene transfer efficiencies. First, new cytokines such as Flt-3 ligand and thrombopoietin, and coculture with stromal elements such as ***fibronectin*** fragments, have been successfully tried during ex vivo culture of HSCs with retroviral vectors. Second, new vectors that meet the host requirements have been developed: pseudotyped retroviral vectors and ***lentiviral*** vectors. Finally, pos. selection of transduced cells has been designed in vitro before reinfusion or in vivo after engraftment to compensate for the low transduction efficiency of HSCs. A novel method of in vivo expansion of transduced hematopoietic cells using the selective amplifier gene may also help overcome the low transduction efficiency of HSCs. It has recently been reported that immunol. tolerance against xenogeneic gene products can be induced by introduction of their genes into HSCs. This distinctive feature further enhances the value of HSCs as a target of gene therapy.
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 6 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1999312774 EMBASE
TI Gene therapy using hematopoietic stem cells.
AU Kohn D.B.
CS D.B. Kohn, Univ. of S. California Sch. of Med., Division of Research Immunology, Children's Hospital Los Angeles, 4650 Sunset Blvd, Los Angeles, CA 90027, United States. dkohn@chla.usc.edu
SO Current Opinion in Molecular Therapeutics, (1999) 1/4 (437-442).
Refs: 53
ISSN: 1464-8431 CODEN: CUOTFO
CY United Kingdom
DT Journal; General Review
FS 022 Human Genetics
LA English
SL English
AB While gene therapy using hematopoietic stem cells was the first area of investigation in the field, success has proven elusive. However, significant progress has been achieved recently in methods for more effective gene transfer and expression. In addition to greatly improved results using retroviral vectors, adeno-associated vectors and ***lentiviral*** vectors appear to be promising for stable transduction of hematopoietic stem cells. These advances, documented in animal transplant models, are now being applied to clinical trials.

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L2 2 S LENTIVIR? AND PRE-STIMULAT? AND STEM CELL
L3 195 S LENTIVIR? AND STEM CELL
L4 8 S L3 AND FIBRONECTIN
L5 6 DUP REM L4 (2 DUPLICATES REMOVED)

=> dup rem l3
PROCESSING COMPLETED FOR L3
L6 149 DUP REM L3 (46 DUPLICATES REMOVED)

=> s l6 and py<1999
1 FILES SEARCHED...
L7 35 L6 AND PY<1999

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L7 ANSWER 1 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:111568 BIOSIS
DN PREV199900111568
TI Transplantation of immunoselected CD34+ cells transduced with a EGFP-expressing ***lentiviral*** vector in non-human primates.
AU Donahue, R. E. (1); An, D. S.; Wersto, R. P.; Agricola, B. A.; Metzger, M. E.; Chen, I. S. Y.
CS (1) Hematol. Branch, NHLBI, Rockville, MD USA
SO Blood, (***Nov. 15, 1998***) Vol. 92, No. 10 SUPPL. 1 PART 1-2, pp. 383B.
Meeting Info.: 40th Annual Meeting of the American Society of Hematology Miami Beach, Florida, USA December 4-8, 1998 The American Society of Heamatology
. ISSN: 0006-4971.
DT Conference
LA English

L7 ANSWER 2 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:108363 BIOSIS
DN PREV199900108363
TI ***Lentiviral*** -based gene transfer of green fluorescence protein into human megakaryocyte progenitor cells.
AU Lebeurier, I.; Martin, T. G.; Shuman, M. A.
CS Hematol.-Oncol. Dep., Univ. Calif. San Francisco, San Francisco, CA USA
SO Blood, (***Nov. 15, 1998***) Vol. 92, No. 10 SUPPL. 1 PART 1-2, pp. 469A.
Meeting Info.: 40th Annual Meeting of the American Society of Hematology

Miami Beach, Florida, USA December 4-8, 1998 The American Society of Heamatology
. ISSN: 0006-4971.
DT Conference
LA English

L7 ANSWER 3 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:38711 BIOSIS
DN PREV199900038711
TI Hypothesis: Myelodysplastic syndromes may have a viral etiology.
AU Raza, Azra (1)
CS (1) Rush Cancer Inst., Rush-Presbyterian-St. Luke's Med. Cent., 2242 W. Harrison St., Suite 108, Chicago, IL 60612-3515 USA
SO International Journal of Hematology, (***Oct., 1998***) Vol. 68, No. 3, pp. 245-256.
ISSN: 0925-5710.
DT General Review
LA English
AB An 'initial transforming event(s)' in a pluripotential bone marrow (BM) ***stem*** ***cell*** confers a growth advantage upon it leading to clonal expansion accompanied by dysplastic maturation resulting in myelodysplastic syndromes (MDS). The nature of this 'initial' event in MDS is obscure. We propose that MDS can begin as a viral disease. It may be a dormant ***lentivirus*** which is made oncogenic by 'promoting events' such as immunosuppression, or a second viral infection. The infected cell may not be a BM ***stem*** ***cell*** , but a cell belonging to the BM stroma or to the immune system. Dysregulated cytokine production as a consequence of the infection can change the BM microenvironment in such a way that optimal growth support is provided only to a rapidly proliferating ***stem*** ***cell*** . Karyotypically marked (or unmarked) abnormal stem cells may exist or arise frequently but do not thrive in a 'normal' cytokine milieu. However, with the changed BM landscape, these abnormal clones may enjoy a growth advantage leading to a monoclonal hypercellular BM and variable cytopenias. Circumstantial evidence to support the possibility that the initial transforming event in MDS is a viral insult is presented in this hypothesis paper.

L7 ANSWER 4 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:519516 BIOSIS
DN PREV199800519516
TI Recent developments in gene therapy for oncology and hematology.
AU Roskrow, M. A. (1); Gaensbacher, B.
CS (1) Institut Experimentalle Chirurgie, Klinikum Rechts Der Isar, Ismaningerstrasse 22, 81675 Muenchen Germany
SO Critical Reviews in Oncology-Hematology, (***Sept., 1998***) Vol. 28, No. 3, pp. 139-151.
ISSN: 1040-8428.
DT General Review
LA English

L7 ANSWER 5 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:479049 BIOSIS
DN PREV199800479049
TI HIV, but not murine leukemia virus, vectors mediate high efficiency gene transfer into freshly isolated G0/G1 human hematopoietic stem cells.
AU Uchida, Nobuko (1); Sutton, Richard E.; Friera, Annabelle M.; He, Dongping; Reitsma, Michael J.; Chang, Wei Chun; Veres, Gabor; Scollay, Roland; Weissman, Irving L.
CS (1) StemCells Inc., 525 Del Rey Ave., Suite C, Sunnyvale, CA 94088 USA
SO Proceedings of the National Academy of Sciences of the United States of America, (***Sept. 29, 1998***) Vol. 95, No. 20, pp. 11939-11944.
ISSN: 0027-8424.
DT Article
LA English
AB Recent studies have opened the possibility that quiescent, G0/G1 hematopoietic stem cells (HSC) can be gene transduced; ***lentiviruses*** (such as HIV type 1, HIV) encode proteins that permit transport of the viral genome into the nucleus of nondividing cells. We and others have recently demonstrated efficient transduction by using an HIV-1-based vector gene delivery system into various human cell types including human CD34+ cells or terminally differentiated neurons. Here we compare the transduction efficiency of two vectors, HIV-based and murine leukemia virus (MuLV)-based vectors, on untreated and highly purified human HSC subsets that are virtually all in G0/G1. The HIV vector, but not MuLV vector supernatants, transduced freshly isolated G0/G1 HSC from mobilized peripheral blood. Single-step transduction using replication-defective HIV resulted in HSC that expressed the green fluorescent protein (GFP) transgene while retaining their ***stem*** ***cell*** phenotype; clonal outgrowths of these GFP+ HSC on bone marrow stromal cells fully retained GFP expression for at least 5 weeks. MuLV-based vectors did not transduce resting HSC, as measured by transgene expression, but did so readily when the HSC were actively cycling after culture in vitro for 3 days in a cytokine cocktail. These results suggest that resting HSC may be transduced by ***lentiviral*** -based, but not MuLV, vectors and maintain their primitive phenotype, pluripotentiality, and at least in vitro, transgene expression.

L7 ANSWER 6 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:318520 BIOSIS
DN PREV199800318520
TI Human immunodeficiency virus type 1 vectors efficiently transduce human hematopoietic stem cells.
AU Sutton, Richard E. (1); Wu, Henry T. M.; Rigg, Richard; Bohnlein, Ernst; Brown, Patrick O.
CS (1) 253 Beckman Cent., Stanford Univ. Med. Cent., Stanford, CA 94305 USA
SO Journal of Virology, (***July, 1998***) Vol. 72, No. 7, pp. 5781-5788.
ISSN: 0022-538X.
DT Article
LA English
AB ***Lentiviruses*** are potentially advantageous compared to oncoretroviruses as gene transfer agents because they can infect nondividing cells. We demonstrate here that human immunodeficiency virus type 1 (HIV-1)based vectors were highly efficient in transducing purified human hematopoietic stem cells. Transduction rates, measured by marker gene expression or by PCR of the integrated provirus, exceeded 50%, and transduction appeared to be independent of mitosis. Derivatives of HIV-1 were constructed to optimize the vector, and a deletion of most of Vif and Vpr was required to ensure the long-term persistence of transduced cells with relatively stable expression of the marker gene product. These results extend the utility of this ***lentivirus*** vector system.

L7 ANSWER 7 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:539510 BIOSIS
DN PREV199699261866
TI Development of HIV vectors for anti-HIV gene therapy.
AU Poeschla, Eric; Corbeau, Pierre; Wong-Staal, Flossie (1)
CS (1) Dep. Med. Biol., Mail Code 0665, Univ. Calif., San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0665 USA
SO Proceedings of the National Academy of Sciences of the United States of America, (1996) Vol. 93, No. 21, pp. 11395-11399.
ISSN: 0027-8424.
DT General Review
LA English
AB Current gene therapy protocols for HIV infection use transfection or murine retrovirus mediated transfer of antiviral genes into CD4+ T cells or CD34+ progenitor cells ex vivo, followed by infusion of the gene altered cells into autologous or syngeneic/allogeneic recipients. While these studies are essential for safety and feasibility testing, several limitations remain: long-term reconstitution of the immune system is not effected for lack of access to the macrophage reservoir or the pluripotent ***stem*** ***cell*** population, which is usually quiescent, and ex vivo manipulation of the target cells will be too expensive and impractical for global application. In these regards, the ***lentivirus*** -specific biologic properties of the HIVs, which underlie their pathogenetic mechanisms, are also advantageous as vectors for gene therapy. The ability of HIV to specifically target CD4+ cells, as well as non-cycling cells, makes it a promising candidate for in vivo gene transfer vector on one hand, and for transduction of non-cycling stem cells on the other. Here we report the use of replication-defective vectors and stable vector packaging cell lines derived from both HIV-1 and HIV-2. Both HIV envelopes and vesicular stomatitis virus glycoprotein G were effective in mediating high-titer gene transfer, and an HIV-2 vector could be cross-packaged by HIV-1. Both HIV-1 and HIV-2 vectors were able to transduce primary human macrophages, a property not shared by murine retroviruses. Vesicular stomatitis virus glycoprotein G-pseudotyped HIV vectors have the potential to mediate gene transfer into non-cycling hematopoietic stem cells. If so, HIV or other ***lentivirus*** -based vectors will have applications beyond HIV infection.

L7 ANSWER 8 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:473831 BIOSIS
DN BA94:105206
TI HEMATOPOIETIC GROWTH FACTORS AS ADJUNCTS TO ANTIRETROVIRAL THERAPY.
AU MILES S A
CS DIV. HEMATOLOGY-ONCOLOGY, UCLA CARE CENTER, ROOM BH-412C CENTER HEALTH SCI., LOS ANGELES, CALIF. 90024-1793.
SO AIDS RES HUM RETROVIRUSES, (1992) 8 (6), 1073-1080.
CODEN: ARHRE7. ISSN: 0889-2229.
FS BA; OLD
LA English
AB Anemia and neutropenia are common complications of HIV infection. Antiretroviral therapy with zidovudine exacerbates bone marrow suppression by inhibiting proliferation of blood cell progenitor cells. In addition, treatment for opportunistic infections or malignancies can involve the use of myelosuppressive drugs. As a consequences, severe anemia and neutropenia can result, thereby limiting the utilization of antiretroviral drugs. Since antiretroviral therapy can increase survial, drugs that ameliorate myelosuppression are important adjuncts in the treatment of HIV-treatment patients. Three hematopoietic growth factors are effective in the treatment of anemia or neutropenia. In four placebo-controlled trials, erythropoietin (EPO) at doses up to 600 U/kg/wk decreased mean transfusion requirements by 37%, increased mean hematocrit by 4.5% and corrected anemia in the majority of patients receiving zidovudine over a 12-week period. In a separate study, granulocyte colony-stimulating factor (G-CSF) corrected leukopenia and isolated neutrophil defects in 22 patients with AIDS without altering HIV expression. When erythropoietin was added to the regimen, combined G-CSF and EPO corrected both anemia and leukopenia and lessened subsequent zidovudine toxicity. Similarly, granulocyte macrophage-colony-stimulating factor (GM-CSF) corrected leukopenia and pre-existing neutrophil defects in patients with HIV infection. In controlled and uncontrolled trials, GM-CSF also appears to reduce toxicity from zidovudine, ganciclovir, and antineoplastic therapy. New combinations of hematopoietic stimulants are being used to decrease the toxicity from combination antiretroviral therapy with alpha interferon and cytotoxic chemotherapy in the treatment of AIDs-related malignancies. Future treatments with additional recombinant cytokines such as human ***stem*** ***cell*** factor (HuSCF) may result in both reduction in myelosuppression from drug therapy, and, possibly, reconstitution of the immune and hematopoietic systems of HIV-infected patients.

L7 ANSWER 9 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:454390 BIOSIS
DN BA94:95790
TI FLUORESCENCE-ACTIVATED SORTING OF TOTIPOTENT EMBRYONIC STEM CELLS EXPRESSING DEVELOPMENTALLY REGULATED LACZ FUSION GENES.
AU REDDY S; RAYBURN H; VON MELCHNER H; RULEY H E
CS CENT. CANCER RES., DEP. BIOL., MASS. INST. TECHNOL., 40 AMES ST., CAMBRIDGE, MASS. 02139.
SO PROC NATL ACAD SCI U S A, (1992) 89 (15), 6721-6725.
CODEN: PNASA6. ISSN: 0027-8424.
FS BA; OLD
LA English
AB Murine embryonic stem (ES) cells were infected with a retrovirus promoter trap vector, and clones expressing lacZ fusion gene (LacZ+) were isolated by fluorescence-activated cells sorting (FACS). Of 12 fusion genes tested, 1 was repressed when ES cells were allowed to differentiate in vitro. Two of three lacZ fusion genes tested were passed into the germ line, indicating the FACS does not significantly affect ***stem*** ***cell*** totipotency. The pattern of lacZ expression observed in vivo was consistent with that seen in vitro. Both fusion genes were expressed in preimplantation blastulas. However, a fusion gene whose expression was unaffected by in vitro differentiation was unbiquitously expressed in day-10 embryos, while the other, which showed regulated expression in vitro, was restricted to cells located along the posterior neural fold, the optic chiasm, and within the fourth ventricle. These results demonstrate the utility of using promoter trap vectors in conjuction with fluorescence sorting to disrupt developmentally regulated genes in mice.

L7 ANSWER 10 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:341891 BIOSIS
DN BR43:31441
TI ***STEM*** ***CELL*** FACTORS STIMULATES IN-VITRO GROWTH OF ERYTHROID PROGENITOR CELLS FROM HIV-POSITIVE PATIENTS.

AU WEINBERG R S; CHUSID E D; GALPERIN Y; CHEUNG T; SACKS H
CS MT. SINAI SCH. MED., NEW YORK, N.Y.
SO THIRTY-SECOND ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL NUTRITION, BALTIMORE, MARYLAND, USA, APRIL 30-MAY 2, 1992. CLIN RES. (1992) 40 (2), 242A.
CODEN: CLREAS. ISSN: 0009-9279.
DT Conference
FS BR; OLD
LA English

L7 ANSWER 11 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:310439 BIOSIS
DN BA94:23589
TI INFLUENCE OF INTERLEUKIN-3 ON ZIDOVUDINE AZT-INDUCED IN-VITRO TOXICITY TO HUMAN HEMATOPOIETIC PROGENITORS.
AU GALLICCHIO V S; HUGHES N K
CS HEMATOL./ONCOL. DIV., LUCILLE P. MARKEY CANCER CENT., 800 ROSE ST., LEXINGTON, KY. 40536-0084.
SO INT J CELL CLONING, (1992) 10 (2), 99-104.
CODEN: IJCCE3. ISSN: 0737-1454.
FS BA; OLD
LA English
AB Zidovudine (AZT), the antiviral drug used in the treatment of acquired immunodeficiency syndrome (AIDS), produces some toxicity to the hematopoietic system. Although several hematopoietic growth factors are currently undergoing clinical trials to evaluate their ability to modulate antiviral toxicity, there are scant data which support their ability to ameliorate AZT toxicity on hematopoietic progenitor cells when combined in vitro. We describe in this report the results of studies designed to evaluate in vitro the capacity of the cytokine interleukin-3 (IL-3), in dose-escalation fashion, to modulate AZT toxicity on normal human marrow derived granulocyte/erythroid/macrophage/megakaryocyte colony-forming units (CFU-GEMM), CFU-granulocyte/macrophage (CFU-GM) and erythroid burst-forming units (BFU-E). Colony formation for each progenitor was increased in the presence of IL-3 compared to cultures plated in its absence. In the presence of AZT (ID50 dose, used for each progenitor), IL-3 reduced AZT toxicity, with the most significant response observed for CFU-GEMM, indicating IL-3 may exert an effect on early, less differentiated hematopoietic progenitors. These studies indicate IL-3 may be an effective agent in reversing the hematopoietic toxicity associated with AZT; however, further in vivo studies are required before clinical use of IL-3 is advocated.

L7 ANSWER 12 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:235441 BIOSIS
DN BA93:123466
TI MODULATION OF HEMATOPOIETIC COLONY FORMATION OF STEM CELLS IN PERIPHERAL
BLOOD BY ANTI-TGF-BETA IN PATIENTS WITH SEVERE IMMUNOSUPPRESSION.
AU HARMS B; KOEGLER G; WERNET P; BRUESTER H T; SCHNEIDER E M
CS INST. BLUTGERINNUNG UND TRANSFUSIONSMED., IMMUNOLOGISCHES LABOR, HEINRICH
HEINE UNIV., MOORENSTRASSE 5, W-4000 DUESSELDORF, FRG.
SO KLIN WOCHENSCHR, (1991) 69 (24), 1139-1145.
CODEN: KLWOAZ. ISSN: 0023-2173.
FS BA; OLD
LA English
AB The influence of transforming growth factor-.beta. (TGF-.beta.) on hematopoiesis has been evaluated by adding blocking antibodies against TGF-.beta. to colony forming assays (CFU-c). When optimum concentrations of recombinant growth factors, granulocyte-macrophage colony stimulating factor (GM-CSF), and interleukin-3 (IL-3) were added to stem cells from the peripheral blood of healthy individuals and certain patients with tumors or HIV infection, the anti-TGF-.beta. capable of blocking 5 ng/ml of active TGF-.beta. had no significant influence on erythroid or myeloid colony formation. However, in certain immunosuppressed individuals, anti-TGF-.beta. resulted in a significant decrease of erythroid colony formation and slight suppression of myeloid colony formation. The significant inhibition of hematopoiesis by plasma of HIV patients could be due to the presence of active forms of TGF-.beta.. The results of the blocking experiments are consistent with the concept that TGF-.beta. in low concentrations is essential for erythropoiesis and myelopoiesis but that the higher levels of TGF-.beta. primarily inhibit erythropoiesis in vitro. TGF-.beta. serves as a coordinating factor when efficient recruitment of granulocytes and monocytes is more essential than erythropoiesis and ***stem*** ***cell*** growth.

L7 ANSWER 13 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:149849 BIOSIS
DN BR42:66049
TI THE ***STEM*** ***CELL*** MAVENS HAD A BLAST THE MOLECULAR BIOLOGY OF HEMATOPOIESIS INNSBRUCK AUSTRIA JULY 14-18 1991.
AU ABRAHAM N G; BENZ E J JR; KARLSSON S; LUTTON J; CLARK S C
CS DEP. MED., NEW YORK MED. COLL., VALHALLA, N.Y.
SO New Biol., (1992) 4 (1), 42-47.
CODEN: NEBIE2. ISSN: 1043-4674.
DT Conference
FS BR; OLD
LA English

L7 ANSWER 14 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:97739 BIOSIS
DN BA93:54289
TI POTENTIAL USE OF HUMAN ***STEM*** ***CELL*** FACTORS AS ADJUNCTIVE THERAPY FOR HUMAN IMMUNODEFICIENCY VIRUS-RELATED CYTOPENIAS.
AU MILES S A; LEE K; HUTLIN L; ZSEBO K M; MITSUYASU R T
CS DEP. MED., DIV. HEMATOLOGY-ONCOLOGY, UCLA AIDS CLINICAL RES. CENTER, ROOM
60-051 CHS, LOS ANGELES, CALIF. 90024-1793.
SO BLOOD, (1991) 78 (12), 3200-3208.
CODEN: BLOOAW. ISSN: 0006-4971.
FS BA; OLD
LA English
AB Hematopoietic dysfunction with peripheral cytopenias is a common complication of human immunodeficiency virus (HIV) infection. Symptomatic anemia is the most common cytopenia and occurs in the presence and absence of myelosuppressive drug therapy such as zidovudine. Drug-induced neutropenia and immune thrombocytopenia are also frequent and occur in up to 50% of acquired immundeficiency syndrome (AIDS) patients. Attempts to reduce the impact of bone marrow failure have focused on dose reduction of zidovudine, ganciclovir, and chemotherapy, and the use of recombinant hematopoietic hormones such as erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF). Despite these maneuvers, approximately 30% of patients with AIDS receiving zidovudine will become transfusion-dependent. This has led to investigations of other cytokines that may increase blood cell formation. The recent identification of decreased number and proliferation of hematopoietic progenitors in patients with HIV infection suggests that agents which have activity on progenitor cell pools may have clinical utility. We demonstrate that human ***stem*** ***cell*** factor (HuSCF) increases burst-forming unit-erythroid (BFU-E), colony-forming unit-granulocyte-monocyte (CFU-GM), and CFU-Mix formation in vitro in normal and HIV-infected individuals. HuSCF also decreases the sensitivity of BFU-E to inhibition by zidovudine without altering HIV replication in lymphocytes or monocytes, altering peripheral blood mononuclear cell proliferation to phytohemagglutinin (PHA) and interleukin-2 (IL-2) or altering the effectiveness of zidovudine or dieoxyinosine in inhibiting HIV replication in lymphocytes or monocytes. These studies suggest that HuSCF may have clinical utility in HIV infection as an adjunctive treatment for HIV-related cytopenias.

L7 ANSWER 15 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1991:484970 BIOSIS
DN BA92:118730
TI EFFECT OF IL-1 IL-6 GM-CSF AND ERYTHROPOIETIN ON THE IN-VITRO TOXICITY ASSOCIATED WITH AZT ON HUMAN BONE MARROW HEMATOPOIETIC PROGENITOR STEM
CELLS CFU-GM AND BFU-E.
AU GALLICCHIO V S; HUGHES N K; HULETTE B C; NOBLITT L
CS HEMATOL./ONCOL. DIV., DEP. MED., LUCILLE P. MARKEY CANCER CENT., 800 ROSE ST. VETERANS ADM. MED. CENT., LEXINGTON, KENTUCKY 40536-0084, USA.
SO ANTIVIRAL CHEM CHEMOTHER, (1991) 2 (2), 75-82.
CODEN: ACCHEH. ISSN: 0956-3202.
FS BA; OLD
LA English
AB The drug azidothymidine (AZT), a synthetic thymidine analogue, has been used in the treatment of acquired immunodeficiency syndrome (AIDS). Clinical use of AZT has induced haematopoietic toxicity manifested by anaemia, neutropenia, and overall bone marrow suppression. Cytokines/growth factors, such as erythropoietin (EPO), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1 (IL-1), interleukin-6 (IL-6), are agents responsible for the growth and regulation of normal haematopoiesis by influencing various classes of haematopoietic progenitors. We report the results of studies designed to investigate the capacity of these factors to influence the toxicity of AZT. Low density, .ltoreq. 1.077 g/cm3, adherent and/or T-cell depleted normal human marrow cells were co-cultured in the presence or absence of AZT and the appropriate growth factor, i.e. EPO for the early erythroid haematopoietic colony-forming progenitor ***stem*** ***cell*** (BFU-E) and GM-CSF for the granulocyte-macrophage haematopoietic colony-forming progenitor ***stem*** ***cell*** (CFU-GM), in dose escalation studies. Additional experiments measured the effect of increasing doses of the cytokines IL-1 and IL-6, alone or in combination in the presence of increasing doses of either EPO or GM-CSF. When comparing the rate of AZT-induced inhibition of BFU-E in vitro, EPO alone (from 2 to 10 U/ml) did not reduce the magnitude of AZT toxicity on BFU-E. GM-CSF alone (up to 1000 U/ml) was ineffective in reversing AZT toxicity on CFU-GM; however, in the presence of either IL-1 and IL-6, AZT toxicity was decreased. These results indicate that certain cytokines/growth factors such as IL-1 or IL-6 in combination with EPO or GM-CSF, but not EPO or GM-CSF alone, may be effective in ameliorating AZT bone marrow toxicity; therefore the use of specific cytokines may be warranted as adjuvant therapy in AIDS.

L7 ANSWER 16 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1991:424632 BIOSIS
DN BR41:74177
TI HUMAN HEMATOPOIETIC ***STEM*** ***CELL*** TOXICITY ASSOCIATED WITH ZIDOVUDINE IN-VITRO EFFECTS OF G-CSF AND M-CSF.
AU GALLICCHIO V; HUGHES N
CS LUCILLE P. MARKEY CANCER CENT., UNIV. KY. MED. CENT., LEXINGTON, KY.
SO ISTITUTO SUPERIORE DI SANITA. VII INTERNATIONAL CONFERENCE ON AIDS: SCIENCE CHALLENGING AIDS; FLORENCE, ITALY, JUNE 16-21, 1991. 464P.(VOL. 1); 460P.(VOL. 2). ISTITUTO SUPERIORE DI SANITA: ROME, ITALY. PAPER. (1991) 0 (0), 149.
DT Conference
FS BR; OLD
LA English

L7 ANSWER 17 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1991:295186 BIOSIS
DN BA92:16201
TI GENE TRANSFER INTO HEMATOPOIETIC STEM CELLS.
AU NIENHUIS A W; MCDONAGH K T; BODINE D M
CS CLINICAL HEMATOL. BRANCH, BUILDING 10, ROOM 7C103, NATIONAL HEART LUNG AND
BLOOD INST., BETHESDA, MD. 20892.
SO CANCER (PHILA), (1991) 67 (10 SUPPL), 2700-2704.
CODEN: CANCAR. ISSN: 0008-543X.
FS BA; OLD
LA English
AB The ability to reliably transfer genes into hematopoietic stem cells with long-term repopulation potential and to selectively express such genes would allow genetic therapy for diseases such as sickle cell anemia and immunologic deficiencies due to T-cell defects, including acquired immune deficiency syndrome (AIDS). Understanding the biology of the hematopoietic ***stem*** ***cell*** is a key element in realizing the full therapeutic potential of gene insertion strategies. Current techniques have efficiency rates of gene insertion of approximately 10% to 20% into murine stem cells and 1% to 5% into primate stem cells. Many challenges, some biologic and some logistic, remain before gene transfer protocols that are successful in the mouse model can be extended to humans.

L7 ANSWER 18 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1991:273873 BIOSIS
DN BA92:6488
TI SMALL NON-CLEAVED-CELL LYMPHOMA UNDIFFERENTIATED LYMPHOMA BURKITT'S TYPE
IN AMERICAN ADULTS RESULTS WITH TREATMENT DESIGNED FOR ACUTE LYMPHOBLASTIC
LEUKEMIA.
AU STRAUS D J; WONG G Y; LIU J; OPPENBERG J; FILIPPA D A; GOLD J W M; OFFIT K; CLARKSON B D
CS MEMORIAL SLOAN-KETTERING CANCER CENTER, 1275 YORK AVENUE, NEW YORK, N.Y.

10021.
SO AM J MED, (1991) 90 (3), 328-337.
CODEN: AJMEAZ. ISSN: 0002-9343.
FS BA; OLD
LA English
AB PURPOSE: Small non-cleaved-cell lymphoma (SNCL) "Burkitt's type," a rapidly growing lymphoma, has been rare among adults in the United States, but has greatly increased in incidence with the acquired immunodeficiency syndrome epidemic. This report details the results of treatment of adult SNCL with a series of protocols originally designed for the treatment of acute lymphoblastic leukemia (ALL). PATIENTS AND METHODS: Between July 1973 and May 1987, 29 adults with newly diagnosed SNCL were treated at Memorial Hospital with intensive chemotherapy originally designed for ALL: the cyclophosphamide L-2, L-10, L-17, and L-20 protocols. Nine patients had positive serologies for human immunodeficiency virus (HIV) infection. One patient with all measurable disease resected was not evaluable for response. RESULTS: Sixteen of 28 evaluable patients (57%) achieved a complete remission with treatment. With follow-up as long as 153 months (median, 47 months), 50% of all patients and 59% of patients with negative or unknown HIV serologies have survived and are probably cured. Patients with an initial serum lactic acid dehydrogenase (LDH) level of greater than 500 U/L had a significantly shortened survival as compared with those with a lower serum LDH. Other pretreatment patient characteristics associated with a shortened survival of borderline statistical significance were high National Cancer Institute stage (C, D) and bone marrow involvement. These results are similar to those for ALL and lymphoblastic lymphoma and are comparable to those for American SNCL in the literature. CONCLUSIONS: Approximately one half of adults with SNCL are curable with intensive chemotherapy. More intensive chemotherapy with hematopoietic growth factor and/or autologous bone marrow or peripheral ***stem*** ***cell*** support may increase curability.

L7 ANSWER 19 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1991:273571 BIOSIS
DN BA92:6185
TI MACROPHAGE-ACTIVE COLONY-STIMULATING FACTORS ENHANCE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION IN BONE MARROW STEM CELLS.
AU KITANO K; ABBOUD C N; RYAN D H; QUAN S G; BALDWIN G C; GOLDE D W
CS DIV. HEMATOL.-ONCOL., ROOM 37-068 CHS, DEP. MED., UCLA SCH. MED., 10833 LE CONTE AVE., LOS ANGELES, CALIF. 90024-1678.
SO BLOOD, (1991) 77 (8), 1699-1705.
CODEN: BLOOAW. ISSN: 0006-4971.
FS BA; OLD
LA English
AB To define the relationship between human immunodeficiency virus type 1 (HIV-1) infection in hematopoietic stem cells and virus production by their progeny, we performed kinetic studies infecting bone marrow (BM) stem cells and culturing them in the presence of hematopoietic growth factors. CD34-positive (CD34+), CD4-negative (CD4-) BM cells were isolated and infected in vitro with the monocytotropic HIV-1JR-FL strain or the laboratory-maintained HTLV-IIIB strain at a high multiplicity of infection. The cells were susceptible to productive infection only with HIV-1 JR-FL, and virus production as measured by p24 protein release was markedly increased (more than fivefold) in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3). Macrophage CSF (M-CSF) was least stimulatory and granulocyte CSF (G-CSF) had no effect on virus production. Virus production coincided with proliferation of mononuclear phagocytes but was not related to granulotypic proliferation in G-CSF-treated BM cultues. Although peak virus production from GM-CSF-treated macrophages occured 2 to 3 weeks after infection, peak virus production in infected stem cells was observed 5 to 6 weeks after. Enhancement in virus production had a more rapid onset when CD34+/CD4- cells were cultured in the presence of both GM-CSF and Il-3 for 7 or 14 days. Under these conditions there was a 10-fold enhancement in virus production after 7 days of preincubation and 1 50-fold enhancement after 14 days. These data indicate that while the ***stem*** ***cell*** compartment may be susceptible to infection with a monocytotropic HIV-1 strain, productive and sustained infection is realized only after macrophage differentiation. The lack of effect of G-CSF on virus production is likely because of the limited effect of this hematopoietin on mononuclear phagocyte generation and function.

L7 ANSWER 20 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1991:228663 BIOSIS
DN BA91:120123
TI IN-VITRO SUPPRESSION OF NORMAL HUMAN BONE MARROW PROGENITOR CELLS BY HUMAN IMMUNODEFICIENCY VIRUS.
AU STEINBERG H N; CRUMPACKER C S; CHATIS P A
CS HARVARD THORNDIKE LABORATORY, CHARLES A. DANA RESEARCH INSTITUTE, DIVISION HEMATOLOGY/ONCOLOGY, 330 BROOKLINE AVENUE, BOSTON, MASS. 02215.
SO J VIROL, (1991) 65 (4), 1765-1769.
CODEN: JOVIAM. ISSN: 0022-538X.
FS BA; OLD
LA English
AB Incubation of normal human nonadherent and T-cell-depleted bone marrow cells with HIVIIIB at multiplicities of infection (MOI) ranging from 0.0001:1 to 1:1 reverse transcriptase (RT) units resulted in the dose-dependent suppression of the in vitro growth of erythroid burst-forming unit (BFU-E), granulocyte-macrophage (CFU-GM), and T-lymphocyte (CFU-TL) colonies of progenitor cells. Maximum inhibition of colony formation was observed at a 1:1 ratio of virus to bone marrow cells. At this MOI, BFU-E and CFU-GM colonies were inhibited by 60 to 80%, while CFU-TL colonies were totally suppressed. Inhibition of colony formation was also observed at an MOI of 0.1:1 but not with further log dilutions of the virus. Incubation of the virus with antibody to gp160 resulted in the complete reversal of ***stem*** ***cell*** suppression and the normalization of colony growth in vitro. For BFU-E and CFU-GM colonies, this reversal was observed with dilutions of antibody up to 1:100 and was no longer observed at titers greater than 1:500. The CFU-TL colony number normalized at titers between 1:10 and 1:50. Human immunodeficiency virus (HIV) also suppressed by 50% the growth of colonies derived from CD34+ ***stem*** ***cell*** fractions. Infection of CD34+ cells and T-cell-depleted, nonadherent cell fractions was demonstrated by detection with HIV-specific DNA probe following amplification by polymerase chain reaction. The results suggest that HIV can directly infect human bone marrow progenitor cells and affect their ability to proliferate and give rise to colonies in vitro. The results indicate a direct role for the virus in bone marrow suppression and a possible mechanism for the cytopenias observed in patients with AIDS.

L7 ANSWER 21 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1991:156517 BIOSIS
DN BA91:82317
TI PROGRAMMED ACTIVATION OF T-LYMPHOCYTES A THEORETICAL BASIS FOR SHORT TERM TREATMENT OF AIDS WITH AZIDOTHYMIDINE.
AU FORSDYKE D R
CS DEP. BIOCHEM., QUEEN'S UNIV., KINGSTON, ONTARIO, CAN. K7L 3N6.
SO MED HYPOTHESES, (1991) 34 (1), 24-27.
CODEN: MEHYDY. ISSN: 0306-9877.
FS BA; OLD
LA English
AB When its T-lymphocyte host cell is activated, the latent (DNA) form of human immunodeficiency virus (HIV) is activated to produce RNA copies which are liberated as virus particles from the cell. In this process the cell is destroyed together with the latent virus. If administered at this time, 3'-azidothymidine (AZT) would specifically prevent the liberated RNA copies replicating and establishing latency in new host cells. The RNA copies would then be degraded by viral or host ribonucleases. Thus, one DNA copy of HIV and its RNA progeny would be eliminated from the body. However, many DNA copies of HIV would remain in other cells. The main problem of therapy with AZT is that activation of host cells to become permissive for production of virus is random in time. Activation depends on chance encounters of an infected person with the particular foreign antigens to which individual T-cells bearing latent HIV can specifically respond. It is primarily for this reason that AZT must be administered continuously. If all T-cells could be polyclonally stimulated at one time, all HIV-bearing T-cells would be destroyed and concomitant administration of AZT for a short term would prevent the replication of all liberated viruses. Unlike most renewable 'end' cells in the body, the maturation of T cell involves processes of positive and negative selection. To preserve the 'educated' T-cell population, T-cell renewal occurs at the end cell, rather than at the ***stem*** ***cell*** level. It is possible that normal physiological signals concerned with this homeostatic regulation of T-lymphocyte population size could be harnessed to produce synchronous activation of all T-lymphocytes. Tumor necrosis factor-alpha, has some of the properties expected of a postulated polyclonal activator needed for this programmed activation of T-lymphocytes.

L7 ANSWER 22 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1991:148616 BIOSIS
DN BR40:68221
TI BONE MARROW TRANSPLANTATION FOR IMMUNODEFICIENCY STATES.
AU PARKMAN R; LENARSKY C; KOHN D; SENDER L; WEINBERG K
CS DIV. RES. IMMUNOL., BONE MARROW TRANSPLANTATION, CHILD. HOSP. LOS ANGELES, DEP. PEDIATR., UNIV. SOUTHERN CALIF., SCH. MED., LOS ANGELES, CALIF. 90027.
SO CHAMPLIN, R. E. AND R. P. GALE (ED.). UCLA (UNIVERSITY OF CALIFORNIA-LOS ANGELES) SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY NEW SERIES, VOL. 137.
NEW STRATEGIES IN BONE MARROW TRANSPLANTATION; SANDOZ-UCLA SYMPOSIUM, KEYSTONE, COLORADO, USA, JANUARY 20-27, 1990. XXIII+457P. WILEY-LISS: NEW YORK, NEW YORK, USA; CHICHESTER, ENGLAND, UK. ILLUS. (1991) 0 (0), 229-236.
CODEN: USMBD6. ISSN: 0735-9543. ISBN: 0-471-56065-0.
DT Conference
FS BR; OLD
LA English

L7 ANSWER 23 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1990:501417 BIOSIS
DN BA90:129763
TI IN-VIVO TOXICITY OF 3' AZIDO-3'-DEOXYTHYMIDINE AZT ON CBA-CA MICE.
AU CRONKITE E P; BULLIS J
CS BROOKHAVEN NATIONAL LAB., MED. DEP., UPTON, LONG ISLAND, N.Y. 11973, USA.
SO INT J CELL CLONING, (1990) 8 (5), 332-345.
CODEN: IJCCE3. ISSN: 0737-1454.
FS BA; OLD
LA English
AB CBA/Ca male mice were given 3'-azido-3'-deoxythymidine (AZT) in drinking water (1 mg/ml) for up to 7 weeks. Water consumption and body weight decreased significantly. Neutropenia and lymphopenia were observed during and after exposure. Significant macrocytic anemia developed and disappeared as a function of red cell life span after stopping AZT intake. A microthrombocytosis was seen. Bone marrow cellularity and spleen colony-forming unit (CFU-s) content fell, but recovered completely and quickly after terminating AZT intake. Hemopoietic ***stem*** ***cell*** function measured by 2 different methods of rescuing fatally irradiated mice was normal 4 weeks after AZT exposure, suggesting that AZT treatment does not induce a long-lasting effect in genetic control of mitotic potential of stem cells. This is in marked contrast to exposure of CBA/Ca mice to benzene and ionizing radiation.

L7 ANSWER 24 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1990:481897 BIOSIS
DN BR39:97258
TI CHARACTERIZATION OF AN HIV-1 INFECTED HL-60 CELL CLONE.
AU BUTERA S T; PEREZ V L; CHAN W C; FOLKS T M
CS RETROVIRUS DIS. BRANCH, DIV. VIRAL RICKETTSIAL DIS., CENT. DIS. CONTROL, ATLANTA, GA. 30333, USA.
SO SYMPOSIUM ON MOLECULAR PATHWAYS OF CYTOKINE ACTION HELD AT THE 19TH ANNUAL UCLA (UNIVERSITY OF CALIFORNIA-LOS ANGELES) SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, PARK CITY, UTAH, USA, JANUARY 27-FEBRUARY 3, 1990. J CELL BIOCHEM SUPPL. (1990) 0 (14 PART B), 47.
CODEN: JCBSD7.
DT Conference
FS BR; OLD
LA English

L7 ANSWER 25 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1990:309091 BIOSIS
DN BA90:28058
TI RETROVIRAL INTEGRATION SITES IN TRANSGENIC MOV MICE FREQUENTLY MAP IN THE VICINITY OF TRANSCRIBED DNA REGIONS.
AU MOOSLEHNER K; KARLS U; HARBERS K
CS HEINRICH-PETTE-INST. EXPERIMENTELLE VIROLOGIE UND IMMUNOLOGIE, UNIV. HAMBURG, MARTINISTRASSE 52, 2000 HAMBURG 20, WEST GERMANY.
SO J VIROL, (1990) 64 (6), 3056-3058.
CODEN: JOVIAM. ISSN: 0022-538X.

FS BA; OLD
LA English
AB Transcription of cellular sequences flanking proviral insertion sites was studied in several Mov mouse strains, each of which carried one copy of the Moloney murine leukemia virus in its germ line. In three out of five randomly chosen Mov strains, the provirus had integrated in the vicinity of DNA regions transcribed in the embryonal ***stem*** ***cell*** line CCE and the embryonal carcinoma cell line F9. Assuming the CCE and F9 cells are developmentally equivalent to the early embryonic cells that were infected to establish the Mov strains, our results suggest that retroviruses integrate preferentially into actively transcribed DNA regions.

L7 ANSWER 26 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1989:418849 BIOSIS
DN BR37:74312
TI BONE MARROW CHANGES IN HIV-1 POSITIVE ASYMPTOMATIC PATIENTS ON ZIDOVUDINE.
AU FANNING M; GELMON K; FALUTZ J; MONTANER J; TSOUKAS C; RUEDY J; ET AL
CS UNIV. TORONTO, BRITISH COLUMBIA.
SO MORISSET, R. A. (ED.). VE CONFERENCE INTERNATIONALE SUR LE SIDA: LE DEFI SCIENTIFIQUE ET SOCIAL; V INTERNATIONAL CONFERENCE ON AIDS: THE SCIENTIFIC AND SOCIAL CHALLENGE; MONTREAL, QUEBEC, CANADA, JUNE 4-9, 1989. 1262P. INTERNATIONAL DEVELOPMENT RESEARCH CENTRE: OTTAWA, ONTARIO, CANADA. ILLUS.
PAPER. (1989) 0 (0), 283.
ISBN: 0-662-56670-X.
DT Conference
FS BR; OLD
LA English

L7 ANSWER 27 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1988:123781 BIOSIS
DN BR34:59643
TI DECREASE OF IN-VITRO COLONY FORMATION OF THE HEMATOPOIETIC PROGENITOR CELLS CFU-GEMM CFU-MK BFU-E AND CFU-GM IN THE ACQUIRED IMMUNODEFICIENCY SYNDROME AIDS.
AU VOELKERS B; GANSER A; STELLA C C; HOELZER D
CS DEP. HEMATOLOGY, UNIV. FRANKFURT, FRANKFURT, FRG.
SO NAJMAN, A., ET AL. (ED.). COLLOQUE INSERM (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE), VOL. 162. LES INHIBITEURS DE L'HEMATOPOIESE; (INSERM (NATIONAL INSTITUTE OF HEALTH AND MEDICAL RESEARCH) COLLOQUIUM, VOL. 162. THE INHIBITORS OF HEMATOPOIESIS); FIRST INTERNATIONAL SYMPOSIUM ON INHIBITORY FACTORS IN THE REGULATION OF HEMATOPOIESIS, PARIS, FRANCE, APRIL 26-28, 1987.. XIX+356P. JOHN LIBBEY EUROTEXT LTD.: MONTROUGE, FRANCE; EDITIONS INSERM: PARIS, FRANCE. ILLUS. PAPER. (1987) 0 (0), 331-334.
CODEN: CINMDE. ISSN: 0768-3154. ISBN: 0-86196-125-0, 2-85598-340-1.
FS BR; OLD
LA English

L7 ANSWER 28 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1987:136302 BIOSIS
DN BR32:64937
TI ALTERATIONS IN THE HEMATOPOIETIC ***STEM*** - ***CELL*** COMPARTMENT IN PATIENTS WITH ACQUIRED IMMUNE DEFICIENCY SYNDROME.
AU VOELKERS B; GANSER A; STELLA C C; HOELZER D
CS DEP. HEMATOL., UNIV. FRANKFURT, FRANKFURT, FRG.
SO ANNUAL MEETING OF THE GERMAN SOCIETY OF HEMATOLOGY AND ONCOLOGY, TUEBINGEN, WEST GERMANY, OCT. 5-8, 1986. BLUT. (1986) 53 (3), 171-172.
CODEN: BLUTA9. ISSN: 0006-5242.
DT Conference
FS BR; OLD
LA English

L7 ANSWER 29 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1986:426723 BIOSIS
DN BR31:92535
TI COMPLETE CORRECTION OF THE ENZYMATIC DEFECT IN GAUCHER DISEASE FIBROBLASTS BY GENE TRANSFER.
AU SORGE J A; WEST W; CRADER W; BEUTLER E
CS SCRIPPS CLIN. RES. FOUND., LA JOLLA, CALIF., USA.
SO SEVENTY-EIGHTH ANNUAL NATIONAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, WASHINGTON, D.C., USA, MAY 2-5, 1986. CLIN RES. (1986) 34 (2), 653A.
CODEN: CLREAS. ISSN: 0009-9279.
DT Conference
FS BR; OLD
LA English

L7 ANSWER 30 OF 35 CAPLUS COPYRIGHT 2002 ACS
AN 1998:794982 CAPLUS
DN 130:21344
TI Mammalian cell transduction for use in gene therapy for hemophilia A
IN Vanden, Driessche Thierry; Chuah, Marinee Khim Lay
PA Leuven Research & Development Vzw, Belg.
SO PCT Int. Appl., 55 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9853063	A2	19981126	WO 1998-EP3013	19980518 <--
WO 9853063	A3	19990318		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
EP 938904	A1	19990901	EP 1998-200382	19980209
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9881057	A1	19981211	AU 1998-81057	19980518 <--
EP 980264	A2	20000223	EP 1998-930718	19980518
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI EP 1997-201480		19970516		
EP 1998-200382		19980209		
WO 1998-EP3013		19980518		

AB The present invention relates to a method for the ex vivo transduction of mammalian cells, in particular to the transduction of bone marrow stromal cells. These cells can be transduced with a gene of interest, in particular a B-domain deleted human factor VIII gene. In the latter case, the transduced cells can be used to treat hemophilia A. The method for the ex vivo transduction of bone marrow stromal cells with the human factor VIII gene comprises provision of an intron-based retroviral vector comprising a B-domain deleted human factor VIII cDNA (designated as MFG-FVIII.DELTA.B); pseudotyping the said vector with the Gibbon ape leukemia virus (GALV) envelope; transducing bone marrow stromal cells with the said pseudotyped vector by pre-incubating the cells for a suitable period of time in cell culture medium without phosphate and subsequently adding a vector-contg. supernatant, optionally supplemented with transduction additives to the cells, followed by centrifuging the mixt. thus obtained; and optionally repeating the two previous steps. An advantage of the method is that no myeloablation is required. Because of this, the gene therapeutic method described is clin. acceptable for hemophilia patients. A large no. of FVIII expressing primary BM stromal cells could be obtained while obviating the need to enrich for transduced cells by selection and without inducing stromal cell proliferation by supplementing high doses of exogenous purified growth factors. These improvements shorten the in vitro culture period of the BM stromal cells that are thus more likely to retain their original properties. Furthermore, since selective enrichment of transduced cells was not needed, it was not necessary to include a neoR selectable marker in the vector.

L7 ANSWER 31 OF 35 CAPLUS COPYRIGHT 2002 ACS
AN 1998:706053 CAPLUS
DN 129:314987
TI Use of ***lentiviral*** vectors for antigen presentation in dendritic cells
IN Wong-Staal, Flossie; Li, Xinqiang; Kan-Mitchell, June
PA The Regents of the University of California, USA
SO PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9846083	A1	19981022	WO 1998-US8313	19980417 <--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9871583	A1	19981111	AU 1998-71583	19980417 <--
EP 1007716	A1	20000614	EP 1998-918708	19980417
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 2001007859	A1	20010712	US 1998-61986	19980417
PRAI US 1997-43264	P	19970417		
WO 1998-US8313	W	19980417		

AB The present invention provides methods for inducing immunity in a subject by using dendritic cells or progenitors transduced with a ***lentivirus*** vector constructed to deliver an antigenic epitope. The methods of the invention are particularly suited to inducing immunity to human immunodeficiency virus (HIV) and other viral diseases, as well as to inducing immunity to tumor antigens.

L7 ANSWER 32 OF 35 CAPLUS COPYRIGHT 2002 ACS
AN 1998:197605 CAPLUS
DN 128:253802
TI Retroviral vectors modified for recognition by the nuclear import system and capable of transducing non-dividing cells
IN Trono, Didier P.; Gallay, Philippe A.
PA Salk Institute for Biological Studies, USA; Trono, Didier P.; Gallay, Philippe A.
SO PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9812314	A1	19980326	WO 1997-US15934	19970908 <--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9742617	A1	19980414	AU 1997-42617	19970908 <--
EP 970201	A1	20000112	EP 1997-940952	19970908
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2001501815	T2	20010213	JP 1998-514725	19970908
PRAI US 1996-715318	A1	19960917		
WO 1997-US15934	W	19970908		

AB In accordance with the present invention, methods have been developed to modify retroviral vectors derived from viruses which are not known to be pathogenic in humans (e.g., murine leukemia virus), so that such vectors are rendered capable of transducing heterologous sequences into non-dividing cells. Thus, it has been discovered that retroviruses can be rendered capable of infecting non-dividing cells by introducing into the viral particle one of several specifically defined modifications. For example, an element which is recognized by the nuclear import machinery of a non-dividing cell can be assocd. with the nucleoprotein complex of the retrovirus. Alternatively, at least one protein encoded by viral gag or pol nucleic acid is modified so as to be recognized by the nuclear import machinery of a non-dividing cell. Integrase is shown to play a dual role in HIV-1 infection of non-dividing cells. First, by binding to the C-terminal phosphotyrosine of matrix protein, integrase mediates the incorporation of the karyophilic properties of matrix protein into the HIV-1 nucleoprotein complex. Second, integrase facilitates the migration

of the viral genome to the nucleopore by interacting with one component of the cell nuclear import machinery, karyopherin .alpha.. Integrase-karyophilin .alpha. complexes in vitro recruit both karyopherin .beta. and nucleoporin, thereby allowing HIV-1 integrase to induce infection of nondividing cells by murine leukemia virus-based vectors. Thus, integrase is a preferred element for use in the practice of the present invention.

L7 ANSWER 33 OF 35 CAPLUS COPYRIGHT 2002 ACS
AN 1997:414200 CAPLUS
DN 127:30124
TI Production of somatic mosaicism in mammals using a gene that can be activated or inactivated by regulatable somatic recombination
IN Federoff, Howard
PA University of Rochester, USA
SO PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9717842	A1	19970522	WO 1996-US18353	19961112 <--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2237392	AA	19970522	CA 1996-2237392	19961112 <--
AU 9711596	A1	19970605	AU 1997-11596	19961112 <--
EP 952787	A1	19991103	EP 1996-942757	19961112
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000500341	T2	20000118	JP 1997-519109	19961112
US 6252130	B1	20010626	US 1996-747328	19961112
US 2001027567	A1	20011004	US 2001-854869	20010514
PRAI US 1995-6822	P	19951113		
US 1996-747328	A1	19961112		
WO 1996-US18353	W	19961112		

AB Methods of inactivating or activating a gene by regulatable somatic recombination are described. One method involves excision of a transcriptional terminator that lies between a promoter and a gene. The terminator is flanked by recombination sites such that when the substrate is treated with a specific recombinase the terminator is excised and gene will be expressed. Constructs can also have a promoter, gene to be controlled, and recombination sites on each side of the gene which when treated with recombinase delete the gene are also provided. Methods of creating transgenic mammals carrying these constructs and inducing somatic recombination are described. The preferred excision mechanism is cre/loxP. An expression construct for the nerve growth factor (NGF) gene that could be activated by excision was prepd. and shown to be a suitable substrate for cre/loxP-mediated excision in Escherichia coli. Transgenic mice carrying the gene were prepd. by microinjection of fertilized eggs. The gene was locally activated in the hippocampus by injecting a herpes simplex virus expression vector for the cre gene into the brain. A local increase in NGF of .apprx.15-fold was found.

L7 ANSWER 34 OF 35 CAPLUS COPYRIGHT 2002 ACS
AN 1997:297375 CAPLUS
DN 126:273247
TI Transformation of quiescent cells by using retroviral system for gene therapy
IN Russell, Stephen James; Fielding, Adele Kay; Casimir, Colin Maurice
PA Medical Research Council, UK; Russell, Stephen James; Fielding, Adele Kay; Casimir, Colin Maurice
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9712049	A1	19970403	WO 1996-GB2405	19960930 <--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG				
CA 2231735	AA	19970403	CA 1996-2231735	19960930 <--
AU 9671379	A1	19970417	AU 1996-71379	19960930 <--
AU 730596	B2	20010308		
EP 856061	A1	19980805	EP 1996-932693	19960930 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001503243	T2	20010313	JP 1997-513235	19960930
PRAI GB 1995-19776	A	19950928		
WO 1996-GB2405	W	19960930		

AB Materials and methods for transferring nucleic acid encoding a polypeptide for treating a disease or disorder into populations of quiescent cells such as hematopoietic stem cells (HSCs), using retroviral packaging cell lines and retroviral particles expressing and displaying a growth factor such as ***stem*** ***cell*** factor (SCF) on the cell surface or as a fusion with a viral envelope protein. The present invention also relates to compns. comprising the retroviral packaging cell lines and retroviral particles, and their use in methods of medical treatment, in vivo and ex vivo.

L7 ANSWER 35 OF 35 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1998236070 EMBASE
TI Identification of a human immunodeficiency virus type 2 (HIV-2) encapsidation determinant and transduction of nondividing human cells by HIV- 2-based ***lentivirus*** vectors.
AU Poeschla E.; Gilbert J.; Li X.; Huang S.; Ho A.; Wong-Staal F.
CS F. Wong-Staal, Department of Medicine 0665, University of California, 9500 Gilman Dr., San Diego, CA 92093-0665, United States. fwongstaal@ucsd.edu
SO Journal of Virology, (1998) 72/8 (6527-6536).
Refs: 66
ISSN: 0022-538X CODEN: JOVIAM
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Although previous ***lentivirus*** vector systems have used human immunodeficiency virus type 1 (HIV-1), HIV-2 is less pathogenic in humans and is amenable to pathogenicity testing in a primate model. In this study, an HIV-2 molecular clone that is infectious but apathogenic in macaques was used to first define cis-acting regions that can be deleted to prevent HIV-2 genomic encapsidation and replication without inhibiting viral gene expression. ***Lentivirus*** encapsidation determinants are complex and incompletely defined; for HIV-2, some deletions between the major 5' splice donor and the gag open reading frame have been shown to minimally affect encapsidation and replication. We find that a larger deletion (61 to 75 nucleotides) abrogates encapsidation and replication but does not diminish mRNA expression. This deletion was incorporated into a replicationdefective, envelope-pseudotyped, three-plasmid HIV-2 ***lentivirus*** vector system that supplies HIV-2 Gag/Pol and accessory proteins in trans from an HIV-2 packaging plasmid. The HIV-2 vectors efficiently transduced marker genes into human T and monocytoid cell lines and, in contrast to a murine leukemia virus-based vector, into growth, arrested HeLa cells and terminally differentiated human macrophages and NTN2 neurons. Vector DNA could be detected in HIV-2 vector-transduced nondividing CD34+ CD38- human hematopoietic progenitor cells but not in those cells transduced with murine vectors. However, stable integration and expression of the reporter gene could not be detected in these hematopoietic progenitors, leaving open the question of the accessibility of these cells to stable ***lentivirus*** transduction.

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FULL ESTIMATED COST		0.00	121.95

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CA SUBSCRIBER PRICE		0.00	-4.34

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=> s RD114 or FLYRD18
L1 261 RD114 OR FLYRD18

=> s l1 and stem cell?
L2 15 L1 AND STEM CELL?

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3 9 DUP REM L2 (6 DUPLICATES REMOVED)

=> s l3 and py<1999
1 FILES SEARCHED...
L4 0 L3 AND PY<1999

=> s l2 and HSC
L5 1 L2 AND HSC

=> d bib abs

L5 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302193 BIOSIS
DN PREV200100302193
TI Multilineage transduction of non-human primate CD34+ hematopoietic cells using RD-114 pseudotyped oncoretroviruses.
AU Kelly, Patrick F. (1); Bonifacino, Aylin C.; Carrington, Jody A. (1); Agricola, Brian A.; Metzger, Mark E.; Kluge, Kim A.; Nienhuis, Arthur W. (1); Donahue, Robert E.; Vanin, Elio F. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 525a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The relative quiescence of the hematopoietic ***stem*** ***cell*** (***HSC***) and the low level of viral receptor expression are known to contribute to the low efficiency of retroviral gene transfer into HSCs of large animals and humans. We have previously reported that ***RD114*** -pseudotyped retroviruses could efficiently transduce cord blood CD34+ cells after 24-48 hours pre-stimulation and a single exposure to the viral particles preloaded onto RetroNectin-coated plates. Based on these results we evaluated gene transfer of ***RD114*** -pseudotyped murine retroviruses using non-human primate CD34+ peripheral blood (PB) cells in the rhesus autologous transplant model. SCF/G-CSF-mobilized rhesus monkey PB were collected and enriched for CD34+ cells. These cells were cultured in serum-containing medium with high concentrations of SCF, FLT-3 and IL-6 and exposed to ***RD114*** -pseudotyped particles preloaded onto RetroNectin-coated plates at 48 hours and 72 hours. After 96 hours in culture, cells were harvested and infused into irradiated recipients (2 X 500 cGy, n=5). The transduction efficiency of the infused cells was 35-55% based on EGFP expression. In all animals we have observed multilineage engraftment with persistence of EGFP expression after 6-8 weeks post-transplantation, a result that was not achieved with a similar construct pseudotyped with the amphotropic envelope protein. In the first animal transplanted, we observed high levels of multilineage engraftment of EGFP+ cells (as high as 98% in granulocytes) over the first 20 weeks post transplantation. After 28 weeks multilineage expression has stabilized at 8-10%. Serial genomic southern analysis for both proviral integrity and integration site indicated that vector silencing was not occurring and that the engraftment of gene modified cells was oligoclonal. The second recipient displayed similar kinetics but died from transplant related complications 8 weeks post-transplantation. Subsequent animals have achieved lower levels of EGFP expression (1-3%) suggesting that transduction conditions using this pseudotype remains to be optimized. These results suggest oncoretroviral vectors pseudotyped with the ***RD114*** envelope protein could be useful for achieving clinically relevant levels of gene transfer into human pluripotent hematopoietic cells.

=> d his

(FILE 'HOME' ENTERED AT 14:22:08 ON 05 FEB 2002)

FILE 'BIOSIS, CAPLUS, EMBASE' ENTERED AT 14:22:23 ON 05 FEB 2002
L1 261 S RD114 OR FLYRD18
L2 15 S L1 AND STEM CELL?
L3 9 DUP REM L2 (6 DUPLICATES REMOVED)
L4 0 S L3 AND PY<1999
L5 1 S L2 AND HSC

=> d bib abs l3 1-
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L3 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2002 ACS
AN 2001:676635 CAPLUS
DN 135:236393
TI Highly efficient gene transfer into human repopulating ***stem*** ***cells*** by ***RD114*** envelope protein pseudotyped retroviral vector particles which pre-adsorb on retronectin-coated plates
IN Kelly, Patrick F.; Vanin, Elio F.
PA St. Jude Children's Research Hospital, USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001066150	A2	20010913	WO 2001-US7212	20010307

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
US 2001051375 A1 20011213 US 2001-801302 20010307
PRAI US 2000-187534 P 20000307
AB The present invention relates to a method for efficiently introducing exogenous genes into ***stem*** ***cells*** , particularly human ***stem*** ***cells*** . The method optionally includes the steps of inducing the proliferation of target cells by pre-stimulation with cytokines and/or growth factors, followed by incubating these cells with ***RD114*** -pseudotyped vector particles. In a specific embodiment, the vector particles are retronectin-immobilized or ultracentrifugation-concd. retroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein. The present invention further discloses a method for somatic gene therapy, which can be used for various therapeutic applications and involves introducing a gene of interest contained within the retroviral genome into human repopulating ***stem*** ***cells*** followed by introducing these cells into a human host. Finally, the present invention discloses a method for monitoring the efficiency of the ***stem*** ***cell*** -mediated gene transfer based on detecting the presence of the genes (or the expression products) of the retroviral vector in various ***stem*** ***cell*** -derived lineages of the host.

L3 ANSWER 2 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:415218 BIOSIS
DN PREV200100415218
TI ***RD114*** -Pseudotyped oncoretroviral vectors: Biological and physical properties.
AU Kelly, Patrick F.; Carrington, Jody; Nathwani, Amit; Vanin, Elio F. (1)
CS (1) Division of Experimental Hematology, Department of Hematology/Oncology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN, 38105; elio.vanin@stjude.org USA
SO Orlic, Donald; Bruemmendorf, Tim H.; Sharkis, Saul J.; Kanz, Lothar. Annals of the New York Academy of Sciences, (June, 2001) Vol. 938, pp. 262-277. Annals of the New York Academy of Sciences. Hematopoietic stem cells 2000: Basic and clinical sciences: Third International Conference. print.
Publisher: New York Academy of Sciences 2 East 63rd Street, New York, NY, 10021, USA.
Meeting Info.: Conference on Hematopoietic Stem Cells: Genetics and Medicine Tubingen, Germany September 14-16, 2000
ISSN: 0077-8923. ISBN: 1-57331-295-9 (cloth), 1-57331-296-7 (paper).
DT Book; Conference
LA English
SL English

L3 ANSWER 3 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2001:526085 BIOSIS
DN PREV200100526085
TI Engraftment of NOD/SCID mice with human CD34+ cells transduced by concentrated oncoretroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein.
AU Gatlin, Joel; Melkus, Michael W.; Padgett, Angela; Kelly, Patrick F.; Garcia, J. Victor (1)
CS (1) Division of Infectious Diseases Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas, Y9.206, Dallas, TX, 75390-9113: victor.garcia@utsouthwestern.edu USA
SO Journal of Virology, (October, 2001) Vol. 75, No. 20, pp. 9995-9999. print.
ISSN: 0022-538X.

DT Article
LA English
SL English
AB Oncoretrovirus vectors pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein produced by the ***FLYRD18*** packaging cell line have previously been shown to transduce human hematopoietic progenitor cells with a greater efficiency than similar amphotropic envelope-pseudotyped vectors. In this report, we describe the production and efficient concentration of ***RD114*** -pseudotyped murine leukemia virus (MLV)-based vectors. Following a single round of centrifugation, vector supernatants were concentrated approximately 200-fold with a 50 to 70% yield. Concentrated vector stocks transduced prestimulated human CD34+ (hCD34+) cells with approximately 69% efficiency (n = 7, standard deviation = 4.4%) using a single addition of vector at a low multiplicity of infection (MOI = 5). Introduction of transduced hCD34+ cells into irradiated NOD/SCID recipients resulted in multilineage engraftment with long-term transgene expression. These data demonstrate that ***RD114*** -pseudotyped MLV-based vectors can be efficiently concentrated to high titers and that hCD34+ cells transduced with concentrated vector stocks retain in vivo repopulating potential. These results highlight the potential of ***RD114*** -pseudotyped oncoretrovirus vectors for future clinical implementation in hematopoietic ***stem*** ***cell*** gene transfer.

L3 ANSWER 4 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:512683 BIOSIS
DN PREV200100512683
TI Sustained multilineage gene persistence and expression in dogs transplanted with CD34+ marrow cells transduced by ***RD114*** -pseudotype oncoretrovirus vectors.
AU Goerner, Martin; Horn, Peter A.; Peterson, Laura; Kurre, Peter; Storb, Rainer; Rasko, John E. J.; Kiem, Hans-Peter (1)
CS (1) Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D1-100, Seattle, WA, 98109-1024: hkiem@fhcrc.org USA
SO Blood, (October 1, 2001) Vol. 98, No. 7, pp. 2065-2070. print. ISSN: 0006-4971.
DT Article
LA English
SL English
AB Previous studies have shown that the choice of envelope protein (pseudotype) can have a significant effect on the efficiency of retroviral gene transfer into hematopoietic ***stem*** ***cells*** . This study used a competitive repopulation assay in the dog model to evaluate oncoretroviral vectors carrying the envelope protein of the endogenous feline virus, ***RD114*** . CD34-enriched marrow cells were divided into equal aliquots and transduced with vectors produced by the ***RD114*** -pseudotype packaging cells FLYRD (LgGLSN and LNX) or by the gibbon ape leukemia virus (GALV)-pseudotype packaging cells PG13 (LNY). A total of 5 dogs were studied. One dog died because of infection before sustained engraftment could be achieved, and monitoring was discontinued after 9 months in another animal that had very low overall gene-marking levels. The 3 remaining animals are alive with follow-ups at 11, 22, and 23 months. Analyses of gene marking frequencies in peripheral blood and marrow by polymerase chain reaction revealed no significant differences between the ***RD114*** and GALV-pseudotype vectors. The LgGLSN vector also contained the enhanced green fluorescent protein (GFP), enabling us to monitor proviral expression by flow cytometry. Up to 10% of peripheral blood cells expressed GFP shortly after transplantation and approximately 6% after the longest follow-up of 23 months. Flow cytometric analysis of hematopoietic sub-populations showed that most of the GFP-expressing cells were granulocytes, although GFP-positive lymphocytes and monocytes were also detected. In summary, these results show that ***RD114*** -pseudotype oncoretroviral vectors are able to transduce hematopoietic long-term repopulating cells and, thus, may be useful for human ***stem*** ***cell*** gene therapy.

L3 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
AN 2001:549788 CAPLUS
TI ***RD114*** -pseudotyped oncoretroviral vectors: Biological and physical properties
AU Kelly, Patrick F.; Carrington, Jody; Nathwani, Amit; Vanin, Elio F.
CS Division of Experimental Hematology, Department of Hematology/Oncology, St. Jude Children's Research Hospital, Memphis, TN, 38101, USA
SO Ann. N. Y. Acad. Sci. (2001), 938(Hematopoietic Stem Cells 2000), 262-277 CODEN: ANYAA9; ISSN: 0077-8923
PB New York Academy of Sciences
DT Journal
LA English
AB Limited functional expression of the viral envelope receptor is a recognized barrier to efficient oncoretroviral mediated gene transfer. To circumvent this barrier we evaluated a no. of envelope proteins with respect to gene transfer efficiency into primitive human hematopoietic ***stem*** ***cell*** populations. We obsd. that oncoretroviral vectors pseudotyped with the envelope protein of feline endogenous virus (***RD114***) could efficiently transduce human repopulating cells capable of establishing multilineage hematopoiesis in immunodeficient mice after a single exposure to ***RD114*** -pseudotyped vector. Comparable rates of gene transfer with amphotropic and GALV-pseudotyped vectors have been reported, but only after multiple exposures to the viral supernatant. Oncoretroviral vectors pseudotyped with the ***RD114*** or the amphotropic envelopes had similar stability in vitro, indicating that the increased efficiency in gene transfer is at the receptor level likely due to increased receptor expression or an increased receptor affinity for the ***RD114*** envelope. We also found that ***RD114*** -pseudotype vectors can be efficiently concd., thereby removing any adverse effects of the conditioned media to the long-term repopulating potential of the target human hematopoietic ***stem*** ***cell*** . These studies demonstrate the potential of ***RD114*** -pseudotyped vectors for clin. use.
RE.CNT 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2000:415630 BIOSIS
DN PREV200000415630
TI Highly efficient gene transfer into cord blood nonobese diabetic/severe combined immunodeficiency repopulating cells by oncoretroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein.
AU Kelly, Patrick F. (1); Vandergriff, Jody; Nathwani, Amit; Nienhuis, Arthur W.; Vanin, Elio F.
CS (1) Division of Experimental Hematology, St Jude Children's Research Hospital, 332 N Lauderdale, Room D-4026, Memphis, TN, 38105 USA
SO Blood, (August 15, 2000) Vol. 96, No. 4, pp. 1206-1214. print. ISSN: 0006-4971.
DT Article
LA English
SL English
AB Limited expression of the amphotropic envelope receptor is a recognized barrier to efficient oncoretroviral vector-mediated gene transfer. Human hematopoietic cell lines and cord blood-derived CD34+ and CD34+, CD38- cell populations and the progenitors contained therein were transduced far more efficiently with oncoretroviral particles pseudotyped with the envelope protein of feline endogenous virus (***RD114***) than with conventional amphotropic vector particles. Similarly, human repopulating cells from umbilical cord blood capable of establishing hematopoiesis in immunodeficient mice were efficiently transduced with ***RD114*** -pseudotyped particles, whereas amphotropic particles were ineffective at introducing the proviral genome. After only a single exposure of CD34+ cord blood cells to ***RD114*** -pseudotyped particles, all engrafted nonobese diabetic/severe combined immunodeficiency mice (15 of 15) contained genetically modified human bone marrow cells. Human cells that were positive for enhanced green fluorescent protein represented as much as 90% of the graft. The use of ***RD114*** -pseudotyped vectors may be advantageous for therapeutic gene transfer into hematopoietic ***stem*** ***cells*** .

L3 ANSWER 7 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302193 BIOSIS
DN PREV200100302193
TI Multilineage transduction of non-human primate CD34+ hematopoietic cells using RD-114 pseudotyped oncoretroviruses.
AU Kelly, Patrick F. (1); Bonifacino, Aylin C.; Carrington, Jody A. (1); Agricola, Brian A.; Metzger, Mark E.; Kluge, Kim A.; Nienhuis, Arthur W. (1); Donahue, Robert E.; Vanin, Elio F. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 525a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The relative quiescence of the hematopoietic ***stem*** ***cell*** (HSC) and the low level of viral receptor expression are known to contribute to the low efficiency of retroviral gene transfer into HSCs of large animals and humans. We have previously reported that ***RD114*** -pseudotyped retroviruses could efficiently transduce cord blood CD34+ cells after 24-48 hours pre-stimulation and a single exposure to the viral particles preloaded onto RetroNectin-coated plates. Based on these results we evaluated gene transfer of ***RD114*** -pseudotyped murine retroviruses using non-human primate CD34+ peripheral blood (PB) cells in the rhesus autologous transplant model. SCF/G-CSF-mobilized rhesus monkey PB were collected and enriched for CD34+ cells. These cells were cultured in serum-containing medium with high concentrations of SCF, FLT-3 and IL-6 and exposed to ***RD114*** -pseudotyped particles preloaded onto RetroNectin-coated plates at 48 hours and 72 hours. After 96 hours in culture, cells were harvested and infused into irradiated recipients (2 X 500 cGy, n=5). The transduction efficiency of the infused cells was 35-55% based on EGFP expression. In all animals we have observed multilineage engraftment with persistence of EGFP expression after 6-8 weeks post-transplantation, a result that was not achieved with a similar construct pseudotyped with the amphotropic envelope protein. In the first animal transplanted, we observed high levels of multilineage engraftment of EGFP+ cells (as high as 98% in granulocytes) over the first 20 weeks post transplantation. After 26 weeks multilineage expression has stabilized at 8-10%. Serial genomic southern analysis for both proviral integrity and integration site indicated that vector silencing was not occurring and that the engraftment of gene modified cells was oligoclonal. The second recipient displayed similar kinetics but died from transplant related complications 8 weeks post-transplantation. Subsequent animals have achieved lower levels of EGFP expression (1-3%) suggesting that transduction conditions using this pseudotype remains to be optimized. These results suggest oncoretroviral vectors pseudotyped with the ***RD114*** envelope protein could be useful for achieving clinically relevant levels of gene transfer into human pluripotent hematopoietic cells.

L3 ANSWER 8 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322016 BIOSIS
DN PREV200100322016
TI Comparison of three retroviral envelopes for high efficiency gene transfer into human marrow mesenchymal cells.
AU Hofmann, Ted J. (1); Capizzani, Tony R. (1); Kelly, Patrick F. (1); Vanin, Elio F. (1); Horwitz, Edwin M. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 220a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB Bone marrow stromal cell (MSCs) are marrow mesenchymal cells that are ideal vehicles for delivery of therapeutic proteins in gene therapy protocols. A major obstacle to any successful gene therapy strategy is obtaining high efficiency transduction of the target cells. To optimize transduction of MSCs for clinical trials, we compared the effect of the retroviral envelope on gene transfer efficiency. Three different pseudotypes of a murine ***stem*** ***cell*** viral vector, encoding the green fluorescent protein (GFP) as a marker, were produced: amphotropic (Ampho) in PA317 cells, GALV in PG13 cells, and ***RD114*** (RD) in ***FLYRD18*** cells. The titer of each supernatant was determined using HeLa cells: Ampho = 4.1 X 104, GALV1 = 3.4 X 103, GALV2 = 1.2 X 105, and RD = 5.0 X 105 tu/ml. Following a standard 3-day transduction protocol, the human MSCs were analyzed by flow cytometry to determine the percentage of GFP positive cells. First, MSCs were transduced with Ampho (MOI = 0.2) yielding 92%; GALV1 (MOI = 0.02), 46%; GALV2 (MOI = 0.6), 68%; and RD (MOI = 2.5), 86% gene transfer. Next, MSCs were transduced with RD at an MOI of 0.2 (equivalent to Ampho) and 83%

gene transfer was observed, not significantly different from the 88% transduction obtained using undiluted RD or the 92% obtained with Ampho. Finally, MSCs were transduced with either Ampho or RD at an MOI of 0.02 (equivalent to GALV1). Ampho transduced 77% and RD 61% of the MSCs, compared to 46% for GALV1. Notably, dilute RD (61%) and dilute Ampho (77%) transduced MSCs as well as the higher titer GALV2 (68%). Northern blot analysis showed an unexpected ratio (8:4:1) for the mRNAs of RDR (***RD114*** receptor), Pit-1 (GALV receptor), and Pit-2 (amphotropic receptor). Although RD and Ampho have similar potential to mediate gene transfer into MSCs, the mRNA for RDR is 8-fold more abundant than Pit-2 mRNA. Further, Pit-1 is 4-fold more abundant than Pit-2 despite the apparent lower gene transfer efficiency. We then compared the standard transduction of MSCs to transduction using RetroNectin coated dishes and found no difference in gene transfer efficiency. We conclude that amphotropic and ***RD114*** pseudotyped vectors are more effective for mediating gene transfer into MSCs. Further, more abundant receptor mRNA does not necessarily indicate a greater potential for transduction by the respective viral pseudotype. A higher titer GALV pseudotyped vector may be adequate for efficient transduction but sufficiently high titer PG13 supernatant has been difficult to generate. Additionally, RetroNectin does not enhance gene transfer in our system. Thus, ***RD114*** or amphotropic envelopes are preferred for clinical trials of MSC gene therapy.

L3 ANSWER 9 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322005 BIOSIS
DN PREV200100322005
TI Sustained multilineage gene persistence and expression in dogs transplanted with CD34+ marrow cells transduced by ***RD114*** pseudotyped oncoretroviral vectors.
AU Horn, Peter A. (1); Goerner, Martin (1); Peterson, Laura (1); Storb, Rainer (1); Kiem, Hans-Peter (1)
CS (1) Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 218a. print. Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology . ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB We have recently reported efficient gene transfer into canine hematopoietic repopulating cells using oncoretroviral vectors pseudotyped by the feline endogenous retrovirus envelope protein (***RD114***). Using a competitive repopulating assay in the dog model we compared gene transfer into hematopoietic ***stem*** ***cells*** between vectors produced by PG13 (GALV pseudotype) and FLYRD (***RD114*** pseudotype). CD34-enriched marrow cells from five dogs were divided into equal aliquots and transduced with LgGLSN (FLYRD), LNX (FLYRD) and LNY (PG13). All three vectors carried the neo gene and short sequence differences that allowed them to be distinguished in a single polymerase chain reaction. The ***RD114*** pseudotyped LgGLSN vector also contained the green fluorescent protein (GFP), enabling us to follow gene expression in transduced cells by flow cytometry. One animal died due to infection before sustained engraftment could be achieved and in the animal with lowest overall transduction rate follow-up was discontinued. We now present follow-up data of three dogs at 9, 18 and 21 months. Up to 10% of peripheral blood cells expressed GFP shortly after transplantation and up to 8% GFP-expressing cells were detected after 21 months. Flow cytometric analysis of hematopoietic subpopulations showed sustained GFP expression in all three dogs in DM5+ granulocytes, CD3+ lymphocytes and CD14+ monocytes. The percentage of GFP expressing cells was higher in granulocytes (up to 8.1%) than in lymphocytes (up to 3.5%) or monocytes (up to 5.7%). Two animals were examined for GFP expression in platelets and were found to have between 1.2-1.3% GFP+ platelets at 9 and at 21 months posttransplant. Since transduction efficiency has been shown to correlate with the level of retroviral receptor expression on target cells, we analyzed expression levels of the ***RD114*** receptor (RDR) on human and dog cells. Northern blot analysis revealed an almost 2-fold higher expression of RDR on human cells suggesting that human cells might be even more susceptible to transduction by ***RD114*** pseudotyped vectors than dog cells. In summary, our data show efficient transduction of canine hematopoietic repopulating cells using ***RD114*** pseudotyped retroviral vectors. The level of gene transfer and the sustained multilineage gene persistence and expression obtained in these experiments suggests that the ***RD114*** pseudotype is a promising alternative pseudotype for human ***stem*** ***cell*** gene therapy.

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FILE 'BIOSIS, CAPLUS, EMBASE' ENTERED AT 14:22:23 ON 05 FEB 2002
L1 261 S RD114 OR FLYRD18
L2 15 S L1 AND STEM CELL?
L3 9 DUP REM L2 (6 DUPLICATES REMOVED)
L4 0 S L3 AND PY<1999
L5 1 S L2 AND HSC

=> s l1 and CD34+
'CD34+' NOT VALID HERE
'CD34+' NOT VALID HERE
'CD34+' NOT VALID HERE

=> s l1 and CD34
L6 13 L1 AND CD34

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7 8 DUP REM L6 (5 DUPLICATES REMOVED)

=> d bib abs

L7 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2002 ACS
AN 2001:676635 CAPLUS
DN 135:236393
TI Highly efficient gene transfer into human repopulating stem cells by ***RD114*** envelope protein pseudotyped retroviral vector particles which pre-adsorb on retronectin-coated plates
IN Kelly, Patrick F.; Vanin, Elio F.
PA St. Jude Children's Research Hospital, USA
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001068150	A2	20010913	WO 2001-US7212	20010307

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2001051375 A1 20011213 US 2001-801302 20010307
PRAI US 2000-187534 P 20000307
AB The present invention relates to a method for efficiently introducing exogenous genes into stem cells, particularly human stem cells. The method optionally includes the steps of inducing the proliferation of target cells by pre-stimulation with cytokines and/or growth factors, followed by incubating these cells with ***RD114*** -pseudotyped vector particles. In a specific embodiment, the vector particles are retronectin-immobilized or ultracentrifugation-concd. retroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein. The present invention further discloses a method for somatic gene therapy, which can be used for various therapeutic applications and involves introducing a gene of interest contained within the retroviral genome into human repopulating stem cells followed by introducing these cells into a human host. Finally, the present invention discloses a method for monitoring the efficiency of the stem cell-mediated gene transfer based on detecting the presence of the genes (or the expression products) of the retroviral vector in various stem cell-derived lineages of the host.

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YOU HAVE REQUESTED DATA FROM 7 ANSWERS - CONTINUE? Y/(N):y

L7 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2001:526085 BIOSIS
DN PREV200100526085
TI Engraftment of NOD/SCID mice with human ***CD34*** + cells transduced by concentrated oncoretroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein.
AU Gatlin, Joel; Melkus, Michael W.; Padgett, Angela; Kelly, Patrick F.; Garcia, J. Victor (1)
CS (1) Division of Infectious Diseases Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas, Y9.206, Dallas, TX, 75390-9113: victor.garcia@utsouthwestern.edu USA
SO Journal of Virology, (October, 2001) Vol. 75, No. 20, pp. 9995-9999. print.
ISSN: 0022-538X.
DT Article
LA English
SL English
AB Oncoretrovirus vectors pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein produced by the ***FLYRD18*** packaging cell line have previously been shown to transduce human hematopoietic progenitor cells with a greater efficiency than similar amphotropic envelope-pseudotyped vectors. In this report, we describe the production and efficient concentration of ***RD114*** -pseudotyped murine leukemia virus (MLV)-based vectors. Following a single round of centrifugation, vector supernatants were concentrated approximately 200-fold with a 50 to 70% yield. Concentrated vector stocks transduced prestimulated human ***CD34*** + (hCD34+) cells with approximately 69% efficiency ($n = 7$, standard deviation = 4.4%) using a single addition of vector at a low multiplicity of infection (MOI = 5). Introduction of transduced hCD34+ cells into irradiated NOD/SCID recipients resulted in multilineage engraftment with long-term transgene expression. These data demonstrate that ***RD114*** -pseudotyped MLV-based vectors can be efficiently concentrated to high titers and that hCD34+ cells transduced with concentrated vector stocks retain in vivo repopulating potential. These results highlight the potential of ***RD114*** -pseudotyped oncoretrovirus vectors for future clinical implementation in hematopoietic stem cell gene transfer.

L7 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:512683 BIOSIS
DN PREV200100512683
TI Sustained multilineage gene persistence and expression in dogs transplanted with ***CD34*** + marrow cells transduced by ***RD114*** -pseudotype oncoretrovirus vectors.
AU Goerner, Martin; Horn, Peter A.; Peterson, Laura; Kurre, Peter; Storb, Rainer; Rasko, John E. J.; Kiem, Hans-Peter (1)
CS (1) Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D1-100, Seattle, WA, 98109-1024: hkiem@fhcrc.org USA
SO Blood, (October 1, 2001) Vol. 98, No. 7, pp. 2065-2070. print.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB Previous studies have shown that the choice of envelope protein (pseudotype) can have a significant effect on the efficiency of retroviral gene transfer into hematopoietic stem cells. This study used a competitive repopulation assay in the dog model to evaluate oncoretroviral vectors carrying the envelope protein of the endogenous feline virus, ***RD114*** . ***CD34*** -enriched marrow cells were divided into equal aliquots and transduced with vectors produced by the ***RD114*** -pseudotype packaging cells FLYRD (LgGLSN and LNX) or by the gibbon ape leukemia virus (GALV)-pseudotype packaging cells PG13 (LNY). A total of 5 dogs were studied. One dog died because of infection before sustained engraftment could be achieved, and monitoring was discontinued after 9 months in another animal that had very low overall gene-marking levels. The 3 remaining animals are alive with follow-ups at 11, 22, and 23 months. Analyses of gene marking frequencies in peripheral blood and marrow by polymerase chain reaction revealed no significant differences between the ***RD114*** and GALV-pseudotype vectors. The LgGLSN vector

also contained the enhanced green fluorescent protein (GFP), enabling us to monitor proviral expression by flow cytometry. Up to 10% of peripheral blood cells expressed GFP shortly after transplantation and approximately 6% after the longest follow-up of 23 months. Flow cytometric analysis of hematopoietic sub-populations showed that most of the GFP-expressing cells were granulocytes, although GFP-positive lymphocytes and monocytes were also detected. In summary, these results show that ***RD114*** -pseudotype oncoretroviral vectors are able to transduce hematopoietic tong-term repopulating cells and, thus, may be useful for human stem cell gene therapy.

L7 ANSWER 4 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 2000:415830 BIOSIS
DN PREV200000415630
TI Highly efficient gene transfer into cord blood nonobese diabetic/severe combined immunodeficiency repopulating cells by oncoretroviral vector particles pseudotyped with the feline endogenous retrovirus (***RD114***) envelope protein.
AU Kelly, Patrick F. (1); Vandergriff, Jody; Nathwani, Amit; Nienhuis, Arthur W.; Vanin, Elio F.
CS (1) Division of Experimental Hematology, St Jude Children's Research Hospital, 332 N Lauderdale, Room D-4026, Memphis, TN, 38105 USA
SO Blood, (August 15, 2000) Vol. 96, No. 4, pp. 1206-1214. print.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB Limited expression of the amphotropic envelope receptor is a recognized barrier to efficient oncoretroviral vector-mediated gene transfer. Human hematopoietic cell lines and cord blood-derived ***CD34*** + and ***CD34*** +, CD38- cell populations and the progenitors contained therein were transduced far more efficiently with oncoretroviral particles pseudotyped with the envelope protein of feline endogenous virus (***RD114***) than with conventional amphotropic vector particles. Similarly, human repopulating cells from umbilical cord blood capable of establishing hematopoiesis in immunodeficient mice were efficiently transduced with ***RD114*** -pseudotyped particles, whereas amphotropic particles were ineffective at introducing the proviral genome. After only a single exposure of ***CD34*** + cord blood cells to ***RD114*** -pseudotyped particles, all engrafted nonobese diabetic/severe combined immunodeficiency mice (15 of 15) contained genetically modified human bone marrow cells. Human cells that were positive for enhanced green fluorescent protein represented as much as 90% of the graft. The use of ***RD114*** -pseudotyped vectors may be advantageous for therapeutic gene transfer into hematopoietic stem cells.

L7 ANSWER 5 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:302193 BIOSIS
DN PREV200100302193
TI Multilineage transduction of non-human primate ***CD34*** + hematopoietic cells using RD-114 pseudotyped oncoretroviruses.
AU Kelly, Patrick F. (1); Bonifacino, Aylin C.; Carrington, Jody A. (1); Agricola, Brian A.; Metzger, Mark E.; Kluge, Kim A.; Nienhuis, Arthur W. (1); Donahue, Robert E.; Vanin, Elio F. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 525a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB The relative quiescence of the hematopoietic stem cell (HSC) and the low level of viral receptor expression are known to contribute to the low efficiency of retroviral gene transfer into HSCs of large animals and humans. We have previously reported that ***RD114*** -pseudotyped retroviruses could efficiently transduce cord blood ***CD34*** + cells after 24-48 hours pre-stimulation and a single exposure to the viral particles preloaded onto RetroNectin-coated plates. Based on these results we evaluated gene transfer of ***RD114*** -pseudotyped murine retroviruses using non-human primate ***CD34*** + peripheral blood (PB) cells in the rhesus autologous transplant model. SCF/G-CSF-mobilized rhesus monkey PB were collected and enriched for ***CD34*** + cells. These cells were cultured in serum-containing medium with high concentrations of SCF, FLT-3 and IL-6 and exposed to ***RD114*** -pseudotyped particles preloaded onto RetroNectin-coated plates at 48 hours and 72 hours. After 96 hours in culture, cells were harvested and infused into irradiated recipients (2 X 500 cGy, n=5). The transduction efficiency of the infused cells was 35-55% based on EGFP expression. In all animals we have observed multilineage engraftment with persistence of EGFP expression after 6-8 weeks post-transplantation, a result that was not achieved with a similar construct pseudotyped with the amphotropic envelope protein. In the first animal transplanted, we observed high levels of multilineage engraftment of EGFP+ cells (as high as 98% in granulocytes) over the first 20 weeks post transplantation. After 28 weeks multilineage expression has stabilized at 8-10%. Serial genomic southern analysis for both proviral integrity and integration site indicated that vector silencing was not occurring and that the engraftment of gene modified cells was oligoclonal. The second recipient displayed similar kinetics but died from transplant related complications 8 weeks post-transplantation. Subsequent animals have achieved lower levels of EGFP expression (1-3%) suggesting that transduction conditions using this pseudotype remains to be optimized. These results suggest oncoretroviral vectors pseudotyped with the ***RD114*** envelope protein could be useful for achieving clinically relevant levels of gene transfer into human pluripotent hematopoietic cells.

L7 ANSWER 6 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:311867 BIOSIS
DN PREV200100311887
TI Improved transduction of human primitive hematopoietic cells with a lentiviral vector pseudotyped with the envelope protein of endogenous feline leukemia virus (***RD114*** .
AU Hanawa, Hideki (1); Kelly, Patrick F. (1); Nathwani, Amit C. (1); Nienhuis, Arthur W. (1); Vanin, Elio F. (1)
CS (1) Division of Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 524a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Conference
LA English
SL English
AB Lentiviral vectors based on HIV have inherent advantages in transducing non-dividing cells in that their pre-integration nucleoprotein complex is relatively stable and able to transverse the nuclear membrane without mitosis. Most HIV based vector systems studied to date have utilized the envelope protein of the vesicular stomatitis virus (VSV-G). We have found that the envelope protein of endogenous feline leukemia virus (***RD114***), when used to pseudotype murine oncoretroviral vectors, yields particles that very efficiently transduce primitive hematopoietic cells from cord blood, including those which establish human hematopoiesis in immunodeficient mice (Kelly et al, Blood 96:1206, 2000). Lentiviral vector particles pseudotyped with ***RD114*** envelope were produced by co-transfecting 293T cells with a vector plasmid which encodes the green fluorescent protein (GFP), a plasmid encoding the HIV matrix and enzyme proteins, a plasmid encoding the HIV tat and rev proteins, and either a plasmid encoding the VSV-G or ***RD114*** envelope protein. Vector production as assessed by p24 measurement in conditioned medium was essentially equivalent (VSV-G = 930ng/ml and ***RD114*** = 1240ng/ml). The titer of VSV-G particles was 30-fold higher on HeLa cells. At a multiplicity of infection (MOI) of 15 (HeLa titers) without prestimulation, transduction of cord blood ***CD34*** + cells averaged 51.5% (range 15-78%) with ***RD114*** pseudotyped HIV vector particles whereas the corresponding values were 5.8% (range 2-9%) with the HIV vector pseudotyped with VSV-G o r less than 1% with murine oncoretroviral vector particles pseudotyped with ***RD114*** . With 48 hours of prestimulation, ***RD114*** pseudotyped lentiviral particles were more efficient than VSV-G pseudotyped particles at transducing cord blood (87% vs. 38%) or peripheral blood (51% vs. 21%) ***CD34*** + cells. Using a second design, cells were exposed to equivalent numbers of vector particles based on p24 measurement. With this design, 72% of cord blood, ***CD34*** + cells and 34% of ***CD34*** +, CD38- cells were transduced with ***RD114*** pseudotyped vector particles compared to 19% and 8%, respectively, with VSV-G pseudotyped lentiviral vector particles. Our results indicate that the ***RD114*** envelope will effectively pseudotype HIV based lentiviral vectors and suggest that ***RD114*** pseudotyped lentiviral vector particles transduce primitive human hematopoietic cells at greater efficiency than do VSV-G pseudotyped lentiviral vector particles.

L7 ANSWER 7 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:322005 BIOSIS
DN PREV200100322005
TI Sustained multilineage gene persistence and expression in dogs transplanted with ***CD34*** + marrow cells transduced by ***RD114*** pseudotyped oncoretroviral vectors.
AU Horn, Peter A. (1); Goerner, Martin (1); Peterson, Laura (1); Storb, Rainer (1); Kiem, Hans-Peter (1)
CS (1) Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA USA
SO Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 218a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DT Article; Conference
LA English
SL English
AB We have recently reported efficient gene transfer into canine hematopoietic repopulating cells using oncoretroviral vectors pseudotyped by the feline endogenous retrovirus envelope protein (***RD114***). Using a competitive repopulating assay in the dog model we compared gene transfer into hematopoietic stem cells between vectors produced by PG13 (GALV pseudotype) and FLYRD (***RD114*** pseudotype). ***CD34*** -enriched marrow cells from five dogs were divided into equal aliquots and transduced with LgGLSN (FLYRD), LNX (FLYRD) and LNY (PG13). All three vectors carried the neo gene and short sequence differences that allowed them to be distinguished in a single polymerase chain reaction. The ***RD114*** pseudotyped LgGLSN vector also contained the green fluorescent protein (GFP), enabling us to follow gene expression in transduced cells by flow cytometry. One animal died due to infection before sustained engraftment could be achieved and in the animal with lowest overall transduction rate follow-up was discontinued. We now present follow-up data of three dogs at 9, 18 and 21 months. Up to 10% of peripheral blood cells expressed GFP shortly after transplantation and up to 8% GFP-expressing cells were detected after 21 months. Flow cytometric analysis of hematopoietic subpopulations showed sustained GFP expression in all three dogs in DM5+ granulocytes, CD3+ lymphocytes and CD14+ monocytes. The percentage of GFP expressing cells was higher in granulocytes (up to 8.1%) than in lymphocytes (up to 3.5%) or monocytes (up to 5.7%). Two animals were examined for GFP expression in platelets and were found to have between 1.2-1.3% GFP+ platelets at 9 and at 21 months posttransplant. Since transduction efficiency has been shown to correlate with the level of retroviral receptor expression on target cells, we analyzed expression levels of the ***RD114*** receptor (RDR) on human and dog cells. Northern blot analysis revealed an almost 2-fold higher expression of RDR on human cells suggesting that human cells might be even more susceptible to transduction by ***RD114*** pseudotyped vectors than dog cells. In summary, our data show efficient transduction of canine hematopoietic repopulating cells using ***RD114*** pseudotyped retroviral vectors. The level of gene transfer and the sustained multilineage gene persistence and expression obtained in these experiments suggests that the ***RD114*** pseudotype is a promising alternative pseudotype for human stem cell gene therapy.

L7 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:46304 BIOSIS
DN PREV200000046304
TI Efficient transduction of ***CD34*** + and ***CD34*** +, CD38- human hematopoietic cells with SCID repopulating cell (SRC) potential with an oncoretroviral vector pseudotyped with a feline endogenous virus (***RD114***) envelope protein.
AU Kelly, Patrick F. (1); Vandergriff, Jody A. (1); Vanin, Elio F. (1); Nienhuis, Arthur W. (1)
CS (1) Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN USA
SO Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 611a. Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology New Orleans, Louisiana, USA December 3-7, 1999 The American

Society of Hematology
ISSN: 0006-4971.
DT Conference
LA English

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L2 15 S L1 AND STEM CELL?
L3 9 DUP REM L2 (6 DUPLICATES REMOVED)
L4 0 S L3 AND PY<1999
L5 1 S L2 AND HSC
L6 13 S L1 AND CD34
L7 8 DUP REM L6 (5 DUPLICATES REMOVED)

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L8 15 L1 AND STEM

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